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CONTENTS

ARTICLES PAGE

A New Standard for the Rapid Detection of DDT Tolerance in *Anopheles quadrimaculatus* Larvae and Pupae ..... Jack Colvard Jones 1

Preliminary Observations on the Relation of Light Trap Collections to Mechanical Sweep Net Collections in Sampling Mosquito Populations ..... G. J. Love and W. W. Smith 9

A Rotary Insect-Preference Trap ..... S. A. Edgar and J. F. Herndon 14

Reconnaissance of Mountain Mosquitoes in the McKinley Park Region, Alaska ..... W. C. Frohne 17

Notes on the Distribution of Mosquitoes in Maryland and Virginia ..... Wm. E. Bickley 22

A Simplified Method for Large-Scale Laboratory Rearing of *Aedes aegypti* (L.) .. J. A. McKiel 25

Winter Breeding of *Culex quinquefasciatus* Say in Columbus, Georgia ..... O. K. Fletcher, Jr., and L. Flanagan 29

Notes on *Aedes triseriatus* Egg Incubation and Colonization .. R. O. Hayes and H. B. Morlan 33

Identification of Blood Meals of Blackflies (Diptera: Simuliidae) Attacking Farm Animals .. A. E. R. Downe and P. E. Morrison 37

Additional Studies on the Distribution of Mosquito Larvae and Pupae Within a Rice Field Check ..... B. G. Markos and E. J. Sherman 40

Tabanidae of the Tennessee Valley Watershed .... W. E. Snow, E. Pickard and J. B. Moore 44

ASSOCIATION NEWS 50

EDITORIAL SECTION 52

NEWS AND NOTES 53

REVIEWS AND ABSTRACTS 58

BIBLIOGRAPHY 59

LIST OF ADVERTISERS 57

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ARTICLES

A NEW STANDARD FOR THE RAPID DETECTION OF DDT TOLERANCE IN *ANOPHELES QUADRIMACULATUS* LARVAE AND PUPAE

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This paper is concerned with defining the levels of tolerance to DDT in larvae and pupae of known sexes, stadial ages, and nutritional and rearing status of a laboratory-reared strain of *Anopheles quadrimaculatus,* as measured by a simple exposure test. This information is compared with data on randomly collected larvae reared under uniform conditions. These data furnish a simple method and a new standard for rapidly and accurately assessing acquired tolerance to DDT. The primary advantage of the test described is that it permits repetitive estimations on the same population. Experiments now in progress indicate that the method can be adapted for other insecticides.

METHODS. The LTD strain of *Anopheles quadrimaculatus* Say (Diptera, Culicidae) was used for all studies. This strain has been bred in the laboratory at the National Institutes of Health for approximately 10 years. For most of this time the mosquitoes were reared under unstandardized conditions. The strain was never knowingly exposed to DDT or any other toxicant.

1. *Rearing conditions.* One hundred newly hatched larvae were counted into each of 4 enamel photographic trays (8″ x 16″ x 2″) and fed daily on finely ground dogfood and brewer's U.S.P. yeast (1:1) until ready for use.

Two hundred twenty pupae were isolated daily for subsequent emergence as adults within 1-cubic-foot, copper-wire-screened, wooden cages. Those remaining were used for non-breeding purposes. Adults were given continuous access to 4 percent dextrose. Four-day-old females were given one hour offerings of male guinea pig blood six days a week until they were discarded at the end of two weeks. Eggs were collected daily from a series of cages, and newly hatched larvae selected usually from a single egg dish.

The insectary was held at 25 ± 2° C. and 70% ± 5% relative humidity.

2. *Sexing and aging of material.* Fourth-stage larvae of known ages were obtained by selecting immediately after their molt from the third stage while the head capsule was still undarkened. They were sexed with 100 percent accuracy by simple microscopic examination of their imaginal antennal discs as previously described by the author in another paper (1957). Male larvae begin to molt to the fourth stage predominantly during the morning on the seventh day after egg deposition. Females molt mainly in the afternoon and evening of that day. Fourth instars of known ages and sex will be referred to hereafter as "regulated" larvae. Those larvae whose sex was not determined and whose ages were only approximately known (± 1 day) will be referred to as "random" larvae.

Third-stage larvae of unknown chronological ages were sexed by differences in external pigmentation as described by Jones (in press), black larvae being about 90 percent females, and light pigmented larvae being 70 to 90 percent males.

[1] Laboratory of Tropical Diseases.

Unless stated otherwise, larvae had access to abundant food prior to and immediately following tests at all times.

Pupae were sexed by examination of their external genitalia. Since pupations occur throughout a day, pupae were picked hourly for precise determinations of age.

The ages of regulated fourth-stage larvae and pupae used in this study are accurate to ± 30 minutes. Each regulated animal was briefly examined microscopically to make sure that it appeared externally normal. Those with any observable abnormality were discarded. Only brief macroscopic observations were made on the appearance of random larvae.

3. *Preparation of the toxicant.* Pure, recrystallized, p,p'DDT was made up in a 1 percent stock solution in pure acetone (Fisher, certified reagent). For each test the stock was syringed directly into distilled water to give a final concentration of 1:10,000.[2] This concentration was employed, because it was desirable to reduce the exposure time as much as possible and still compare larval and pupal tolerance levels. Suspensions were used in preference to emulsions because larvae did not feed on suspensions and the DDT could be more easily washed off treated specimens.

DDT suspensions were never more than 30 minutes old at the beginning of any test. Microscopic examination of such suspensions showed extremely uniform particles which were evenly distributed.

4. *Testing procedure.* Larvae and pupae were rinsed once in distilled water just before placing single individuals in 30 ml. glass beakers containing 10 ml. of DDT 10^{-4}. From 5 to 10 specimens were used in a given test. Larvae were kept under fasting conditions only during the exposure to DDT and subsequent washing. Depending upon their age, larvae were exposed 1, 2½, 5, 10, 15, 20, 30, 45, 60, and 75 minutes. Pupae were exposed 30, 45, 60, 120, 180, and 360 minutes. Exposure times are accurate to ± 2 to 5 seconds. From 5 to 10 replicates were made at each exposure time for each age and sex. After exposure, all animals used in a test were rapidly pooled in a liter of distilled water and given an additional rinse in another liter of water before placing them individually in numbered, 50 ml. glass beakers containing food and water. The condition of the larvae and pupae was checked at 2 hours and daily thereafter until they died, pupated, or emerged, individual records being kept for each animal. Successful emergence terminated the tests and served as check on the accuracy of sexing. Approximately 5,000 specimens were used in this study. All tests were performed in a special insectary held at 25 ± 2° C. and 70 ± 5 percent relative humidity by a single operator.

5. *Statistical procedures.* MLT's, standard errors, 95 percent confidence limits, regression coefficients of probit fit were determined by Karber's method (Finney, 1947).

The data obtained in this study fitted an arithmetical better than a log probit model. The MLT's given are somewhat higher than they would be with a log scale; nevertheless, the statistical relationships are not changed in any important way.

RESULTS. 1. *Data on "regulated" material.* About 87 percent of those larvae which survive 24 hours after exposure to DDT 10^{-4} are able to pupate and successfully emerge as adults as shown in Table 1. The percentage tends to increase for the older fourth instars (trend at the 0.007 level), but there is no trend with length of exposure. The important fact in these data is that an accurate measure of adult survival can be calculated from the 24-hour readings alone.

In tolerance studies, the primary concern is with the number of organisms able to survive a given stress. The data in Table 2 present the percentages of larvae and pupae of different ages and sexes able to survive 24 hours and/or capable

[2] Equals 100 p.p.m.

Table 1.—Observed proportions of *Anopheles quadrimaculatus* larvae surviving 24 hours after DDT exposure which were able to pupate and successfully emerge as adults

Sex and age in 4th stage when exposed		% 24-hour survivors able to pupate and emerge as adults Minutes exposed to DDT									
		1'	2-1/2'	5'	10'	15'	20'	30'	45'	60'	Combined times
1 hr.	♂	77.8	82.1	85.7							80.8
	♀	80.0	83.9	55.5							77.8
24 hr.	♂	78.1	89.3	89.3	94.1	72.7	87.0				84.8
	♀	92.9	96.8	96.3	88.2	75.0	95.8				89.7
48 hr.	♂				93.5	96.7	90.5	75.0			92.2
	♀				94.6	100.0	92.6	70.6	100		92.4
70 hr.	♂							96.6	100	85.7	96.2
	♀							100.0	100	100	100.0
											87.0

TABLE 2.—Mean data on percentage of DDT-exposed *Anopheles* surviving at 24 hours and percentage able to develop into adults.

Stage and age when tested	Point of survival	Minutes exposed to DDT 10^{-4}											
		1'		2-1/2'		5'		10'		15'		20'	
		♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Third stage	24 hr S 1/	92.0	94.2	97.1	97.5	85.0	92.3	63.4	74.6	44.4	53.6	28.6	26.0
	Adult 2/	81.3	87.5	94.3	90.0	75.0	87.2	73.2	59.3	33.3	48.7	22.4	15.0
Fourth stage													
1 hr. old	24 S	97.3	83.3	77.8	96.8	28.6	33.3	2.6	5.6				
	Adult	75.7	66.7	63.9	81.3	24.5	18.2	2.6	2.7				
24 hr. old	24 S	97.0	96.6	100.0	100.0	90.3	75.0	97.1	81.0	60.0	90.9	51.1	63.2
	Adult	75.7	89.7	89.3	96.8	80.6	72.2	91.5	71.4	43.6	68.2	44.4	60.5
48 hr. old	24 S							98.9		90.9	87.5	75.0	87.1
	Adult							92.0	89.7	87.9	87.5	67.9	80.6

1/ Percent surviving 24 hours after exposure.

2/ Percent surviving to adult stage.

TABLE 2.—(Continued)

Stage and age when tested	Point of survival	Minutes exposed to DDT 10^{-4}													
		30'		45'		60'		75'		120'		180'		360'	
		♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Third stage	24 hr S1/	.7	.7												
	Adult2/	0	.7												
Fourth stage															
24 hr. old	24 S	5.4	9.1	0	0										
	Adult	3.6	9.0	0	0										
48 hr. old	24 S	28.6	54.8	7.5	23.7	1.1	18.2								
	Adult	21.4	38.7	8.7	23.7	1.1	7.7								
70 hr. old	24 S	82.9	93.8	29.3	48.4	9.9	29.0	6.8	0						
	Adult	80.0	93.8	29.3	48.4	8.5	29.0	6.8	0						
Pupal stage															
1 hr. old	Adult	68.2	91.7	64.0	72.9	52.5	44.1			12.0	30.0	2.9	5.6	2.2	0
24 hr. old	Adult	91.0	97.4	94.6	78.6	75.8	65.9			70.4	63.4	32.5	72.9	13.2	12.5

1/ Percent surviving 24 hours after exposure.

2/ Percent surviving to adult stage.

TABLE 3

Stage and/or age when exposed	Median lethal time of exposure to DDT 10^{-4}, with 95% confidence limits							
	In 24 hours				Before adult emergence			
	♂		♀		♂		♀	
	MLT	95% limits	MLT	95% limits	MLT	95% limits	MLT	95% limits
3rd stage	14	13-16	16	14-17	13	12-15	12	11-13
4th stage								
1 hour	4.5	3.7-5.3	5	4.2-5.8	3.8	3.1-4.4	3.8	3.3-4.2
24 hour	19.5	17-21	21	19-23	17	15-19	19	17-21
48 hour	27.5	25-30	36	32-40	26	23-28	32	28-36
70 hour	42	39-45	48	44-52	43	40-45	48	44-52
Pupal stage								
1 hour	-	-	-	-	76	74-79	87	84-89
24 hour	-	-	-	-	184	181-187	220	216-223

of emerging as adults after exposure to fresh DDT acetone suspensions.

From the data in Table 2, median lethal times of exposure (MLT) (in minutes) with 95 percent confidence limits were calculated as found in Table 3. The standard errors multiplied by the square root of n (n = number of larvae in a test series at each exposure time) and probit line data for the MLT's are given in Table 4.

there is a four- to fivefold increase within 24 hours in the fourth stadium and that as these larvae grow older they progressively increase in their ability to withstand DDT exposures until just before pupation they have developed a nine- to twelve-fold increase in tolerance. As soon as they pupate, there is an additional 1.8 fold increase and within 24 hours some pupae are able to withstand a 6-hour exposure to DDT 10^{-4}. Once pupation occurs, the

TABLE 4.—Further calculations on 24-hour MLT data (see Tables 2 and 3)

Stage	SE $\sqrt{n-1}$ (n = number in group)		Probit line			
			intercept		slope of line	
	♂	♀	♂	♀	♂	♀
3rd stage	5.058	4.122	5.55	5.67	.038	.043
4th stage						
1 hour	2.522	2.187	6.83	6.77	.409	.354
24 hour	5.883	5.480	7.41	7.16	.124	.103
48 hour	7.904	11.109	7.53	7.02	.092	.056
70 hour	10.154	10.564	9.10	8.45	.097	.071

One of the major facts brought out in these tables is that the sex of the larvae does not significantly affect the tests. In other words, male and female larvae of the same ages are essentially equal in their responses to DDT exposure tests.

Relative tolerance levels of different preimaginal stages of *Anopheles quadrimaculatus* to DDT 10^{-4} may now be estimated from Table 3 so that an over-all picture may be obtained. By comparing the MLT's of 1-hour-old fourth instars with others in that stadium and with other stages, it may be seen in Table 5 that

females are significantly more tolerant than males, but the percentage sexual differences do *not* grow significantly more pronounced with age of the pupae.

The data that have been presented provide a uniform and simple standard for detecting with considerable accuracy the levels of DDT tolerance in larvae and pupae of *Anopheles quadrimaculatus*. Levels outside the 95 percent confidence levels given in Table 3 would constitute significant alterations in tolerance to DDT.

2. *Data on random larvae.* It seemed especially desirable to perform these tests

TABLE 5.—Relative DDT tolerances in larval and pupal stages of *Anopheles quadrimaculatus*

Stage	Degrees of tolerance relative to 1-hour old fourth stage larvae			
	♂		♀	
	at 24 hrs.	at adult emergence	at 24 hrs.	at adult emergence
3rd larval	3.1	3.4	3.2	3.2
4th larval				
24 hours	4.2	4.5	4.2	5.0
48 hours	6.0	6.8	7.2	8.4
70 hours	9.3	11.3	9.6	12.6
Pupal stage				
1 hour	...	20.0	...	22.9
24 hours	...	48.4	...	57.9

with randomly selected larvae (grown under uniform conditions) whose ages were only roughly known (± 1 day) and whose sex was not determined, for then exposure testing might well be valuable in practical field tests for measuring tolerance in preimaginal stages of *Anopheles* mosquitoes.

On the eighth and ninth day after egg deposition, fourth instars of undetermined sexes were subjected to the exposure test and their MLT's calculated. These determinations were corrected by Abbott's formula for the percentage normally failing to pupate. The MLT for 8-day-old random larvae (approximately 12-hour-old fourth instars) is approximately 13 minutes (95 percent confidence limits 10–16). This value matches reasonably well the mean MLT of 10.9 minutes obtained from 1- to 24-hour-old fourth instars in Table 3. The MLT for 9-day-old random larvae (approximately 36-hour-old fourth instars) is 27 minutes (95 percent limits 23–30), which compares favorably with the mean MLT of 23.5 of 24- to 48-hour-old fourth instars of Table 3.

Two preliminary tests were made with 8- and 9-day-old random fourth instars to see whether feeding and overcrowding during exposure to DDT would affect the MLT. As Table 6 shows, neither of these factors significantly alters this value.

TABLE 6.—MLT's when 8- and 9-day-old random larvae were either fed or overcrowded during DDT exposure tests

Treatment		MLT minutes	95% confidence limits
8-day-old	Feeding during exposure	11	8–14
	Fasting during exposure	13	10–16
9-day-old	Crowded (5/beaker)	23	22–26
	Not crowded (1/beaker)	27	23–30

To determine what effect starvation would have, newly molted fourth-stage larvae of known sexes were made to fast for 24 hours and were then exposed individually to DDT. Twenty-four-hour-old starved larvae had strikingly lowered tolerances (MLT of 3 minutes, 95 percent limits 1.2–4.8). Starvation thus reduced tolerance levels to that of 1-hour-old fourth instars.

DISCUSSION. The data presented show (1) that DDT tolerance in *Anopheles* larvae reaches its lowest level after the molt, (2) that as larvae grow within the fourth stadium these levels significantly rise day by day, (3) that sexual differences in the larval stages are of no significance, (4) that newly metamorphosed pupae are approximately twice as tolerant to DDT as the most tolerant larvae, (5) that significant sexual differences appear first in the pupal stage, and (6) that while pupal tolerance also significantly increases with age, the differences between sexes do not grow greater.

The DDT exposure test described in this paper is rapid, easy to perform, requires a minimum of equipment, and can be used to provide laboratory standards of considerably wider range and greater accuracy than are now available with continuous exposure testing methods. Tests on random larvae reared under uniform conditions give sufficiently rapid and accurate data to suggest that it may have value in practical field testing for tolerance. The test has the special advantage of permitting as many as 6 laboratory determinations and at least 4 field determinations on the same population.

SUMMARY. 1. A simple exposure test is described for rapidly and accurately measuring DDT tolerance in larvae and pupae of *Anopheles quadrimaculatus*. This technique permits as many as 6 laboratory and 4 field determinations of resistance on a given population and may have considerable practical importance.

2. Different levels of tolerance to DDT in third and fourth stage larvae and pupae of *A. quadrimaculatus* are defined according to exact stadium age and sex.

3. Newly molted fourth-stage larvae obtain lethal doses of DDT significantly more rapidly than either third or older fourth instars.

4. A nine- to twelvefold increase in DDT tolerance occurs during the fourth stadium.

5. With pupation there is a further increase in tolerance and 1-day-old pupae are 48 to 58 times more tolerant than 1-hour-old fourth instars.

6. Female and male larvae of the same chronological ages have essentially equal levels of DDT tolerance, but once pupation occurs females are significantly more tolerant than males.

7. Valid data may be obtained with randomly collected larvae provided their stadium ages are known to roughly 1 day.

8. Tests on random larvae indicate that feeding or overcrowding during exposure testing do not significantly alter results. Starvation of larvae for 24 hours greatly reduces their tolerance.

Acknowledgments. I am greatly indebted to Mary M. Wilkinson for much statistical help and advice throughout this study.

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PRELIMINARY OBSERVATIONS ON THE RELATION OF LIGHT TRAP COLLECTIONS TO MECHANICAL SWEEP NET COLLECTIONS IN SAMPLING MOSQUITO POPULATIONS

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Introduction. Light traps have been used extensively to collect mosquitoes, but in many analyses of data thus obtained variations in attractivity of light to different species of mosquitoes have not been considered. The present study was undertaken to compare, both qualitatively and quantitatively, collections of mosquitoes from light traps and from a non-attractive device.

Materials and Methods. Collections were made at weekly intervals from April 1954 through December 1955. Three New Jersey light traps equipped with 25-watt frosted incandescent bulbs were operated one night each week at 6-, 25-, and 40-foot elevations, respectively, on a tower in a wooded area (Fig. 1). On a different night each week, 6 pairs of mechanical sweep nets were operated from the tower at elevations of 3, 6, 15, 25, 40, and 50 feet. The sequence in which the traps and nets were used was alternated weekly. Data from 60 weeks when all series of traps operated successfully are considered in this report.

One of the mechanical sweep nets is shown in Fig. 2. The opening of the net, formed of a hoop of ¼-inch iron rod, was 18 inches in diameter. Attached to the hoop was an 18-mesh screen wire cone 24 inches long reinforced by ½-inch hardware cloth. The apex of the cone was truncated and a metal strip was attached

FIG. 1. Tower at Mossy Pond, Baker County, Georgia, used in study of vertical distribution of mosquitoes.

Fig. 2. Mechanical sweep net with cage attached.

to form a collar that would fit snugly inside the smaller opening of the holding cage. The cage for retaining and protecting mosquitoes was attached to the cone by two spring hooks. This cage consisted of two tin cans, one number 10 and one number 2 (Fig. 3). A hole, the diameter of the smaller can, was cut in the center of the bottom of the larger can. Both ends were removed from the smaller can and the rim of the cylinder was flared slightly to enable a snug fit when the small can was inserted in the opening of the large can. The small can was held in place by soldering the flared edge to the bottom of the large can. A screened top covered the opening of the large can. Since the height of the number 2 can is less than that of the number 10 can, mosquitoes could fly from the air stream to more protected parts of the cage. Nets with openings toward opposite directions were welded to the ends of a ½-inch iron rod 7 feet in length. Rods with nets attached were affixed to a ¾-inch iron rod 3 feet long held vertical on a stationary

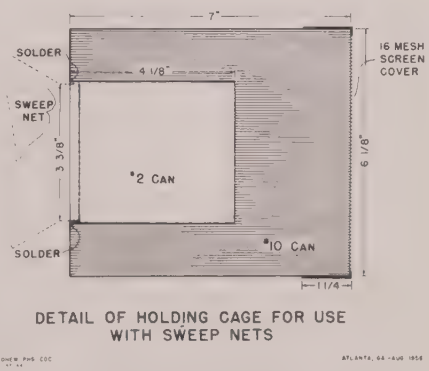

Fig. 3. Diagram of holding cage for mosquitoes caught by sweep nets.

support. The cones were rotated with axes parallel to the ground surface at approximately 25 r.p.m. Power was obtained by a belt and pulleys attached to a vertical ¾-inch iron rod 50 feet in length which was driven by a one horsepower electric motor at the 15-foot level. Sweep nets and light traps were controlled by automatic switches.

The light traps were operated from 6 p.m. to 6 a.m. To prevent escape of mosquitoes from the cages, sweep nets were operated from 6 p.m. until stopped manually around 9 a.m. the following morning. All specimens were identified except those in exceptionally large catches, in which case samples not less than one-eighth of the total catch were identified. Results of counts and identifications were recorded on field sheets and later transferred to mechanical record cards. Data on spatial distribution and activity patterns of various species of mosquitoes are to be reported elsewhere (Love and Platt, manuscript).

The index of attractiveness of light traps for any species was obtained by dividing the total number of specimens caught in the light traps by the total number taken in the mechanical sweep nets.

RESULTS. Table 1 shows results of the study. In the upper portion of the table indices of attractiveness to light traps are listed in descending order for all species of mosquitoes where at least 100 specimens were collected by each method. *Uranotaenia sapphirina,* index 7.67; *Anopheles crucians,* index 6.21; and *Aedes vexans,* index 3.90, showed the greatest attraction to light traps. *Aedes infirmatus,*

TABLE 1.—Species and numbers of mosquitoes caught in mechanical sweep nets and light traps, and indices * of light trap attractiveness: Baker County, Georgia; April 1954–December 1955.

Species	Sweep Nets: Number of Specimens	Light Traps: Number of Specimens	Index
Uranotaenia sapphirina	1,488	11,417	7.67
Anopheles crucians	564	3,505	6.21
Aedes vexans	4,845	18,893	3.90
Culex (Melanoconion) spp.	295	753	2.55
Anopheles quadrimaculatus	282	703	2.40
Aedes atlanticus	113	259	2.29
Culex territans	125	161	1.29
Culex restuans	141	180	1.28
Aedes infirmatus	342	343	1.00
Psorophora ferox	529	128	0.24
Culiseta inornata	24	457	19.04
Aedes mitchellae	15	121	8.07
Anopheles punctipennis	5	34	6.80
Psorophora confinnis	27	171	6.33
Psorophora ciliata	7	30	4.29
Psorophora discolor	3	8	2.67
Mansonia perturbans	73	193	2.64
Culex quinquefasciatus	44	55	1.25
Culiseta melanura	34	42	1.24
Aedes fulvus pallens	13	14	1.08
Psorophora varipes	2	2	1.00
Culex nigripalpus	21	19	0.90
Orthopodomyia signifera	10	8	0.80
Aedes dupreei	77	52	0.68
Aedes thibaulti	8	4	0.50
Psorophora cyanescens	3	1	0.33
Aedes triseriatus	48	10	0.21

* The index of light trap attractiveness is statistically unreliable for the species of mosquitoes listed below the double line due to the small numbers collected by either or both methods.

index 1.00, had little attraction to light traps. The *Culex (Melanoconion)* spp. group, *Anopheles quadrimaculatus,* and *Aedes atlanticus* had about equal indices: 2.55, 2.49, and 2.29 respectively. *Culex territans* and *C. restuans,* indices 1.29 and 1.28 respectively, had little response to light traps. Some species in the same genus varied greatly in response to light traps. Three species of *Culex—territans, restuans,* and *quinquefasciatus*—responded similarly; all three showed a weak attraction to light traps.

The mosquitoes listed below the double line in Table 1 were collected in numbers believed too small to provide statistically valid indices. These indices may be changed when additional data become available. Indications are that *Culiseta inornata,* tentative index 19.04, probably will exhibit a very high index, perhaps the highest of all species studied. *Aedes mitchellae, Psorophora confinnis,* and *Anopheles punctipennis* are known to be strongly attracted to lights and will likely exhibit high indices.

Discussion. The results reported here are based upon 60 weekly collections well distributed throughout the mosquito breeding seasons of 2 years when conditions of drought prevailed. The distribution and density of species are not regarded as characteristics of years when different climatic conditions prevail. Annual precipitation in 1954 was 27.13 inches (27.1 inches below average) and 39.64 inches in 1955 (14.5 inches below average). Consequently, species which breed in relatively permanent ponds were scarce. During spring and early summer of 1954 some of the comparatively permanent ponds were partially filled and most species that develop in such habitats were collected during this period. *Mansonia* spp. were collected at this time but not during 1955 after the complete drying of breeding areas late in 1954. The sweep nets have the advantage of sampling mosquitoes from an area of known extent. Since sweep nets probably are not attractive to mosquitoes and, consequently, catch only the specimens which happen to fly into the path of the nets in operation, specimens should be collecetd in numbers approximating their actual areal density at the time of collection. In contrast, light traps may positively attract phototactic species from a considerable area and negatively phototactic species may be collected in disproportionate numbers.

For example, indices for *Culiseta inornata* and *Psorophora ferox* were 19.04 and 0.24. The latter species is usually rare and the former relatively common in collections with light traps. With sweep nets, however, several times as many *P. ferox* as *C. inornata* were collected.

Comparison of relative density of species on the basis of collections from light traps may be misleading. For example, approximately 5 times as many *Anopheles crucians* as *A. quadrimaculatus* were collected in light traps. In sweep nets, however, the ratio of *A. crucians* to *A. quadrimaculatus* was only 2:1.

In spite of the limitations of light traps, they are probably the best single method for sampling the mosquito population of an area. In studies on ecology of disease, however, precise expression of vector density is essential for evaluation of transmission potential. Collections from light traps do reflect fluctuations in populations of some mosquitoes about as well as other methods (Love, Goodwin, and Smith, manuscript). Although various attempts have been made to determine the abundance of mosquitoes in an area, using the Lincoln index or similar methods, no practical means are available for estimating the actual abundance of mosquitoes.

The objective of the present study is to provide a method for applying data from light trap collections to indicate more accurately the actual species composition and comparative density of a local mosquito fauna. Other types of collecting devices might provide indices of different magnitude. Since all species would be similarly affected, however, the relationships between the index numbers would be the same.

SUMMARY. Procedures are described which enable interpretation of data from light trap collections to indicate more accurately the actual species composition and comparative density of mosquitoes.

On different nights each week vertical series of light traps and mechanical sweep nets were operated in a wooded area near a mosquito breeding pond during 2 mosquito breeding seasons in southwestern Georgia. Indices of the attractiveness of light traps for 27 species of mosquitoes were determined by dividing the numbers of mosquitoes of each species taken in the light traps by the numbers taken in the sweep nets.

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A ROTARY INSECT-PREFERENCE TRAP

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One of the problems confronting the investigator who is studying certain aspects of the bionomics of flying insects with the aid of preference traps is that of eliminating the bias of position. A review of the literature revealed that most traps that had been described were limited in their use (Peterson, 1953). Several individual stationary traps or a single direction trap with several compartments may offer unequal attraction to insects because of unequal distances of the traps from the source of insects or because of wind direction, adjacent trees and rough terrain.

Investigations at this station had revealed that *Culex quinquefasciatus* fed readily on chickens and that it preferred chicken blood to that of all other domestic animals tested (Edgar, *et al.,* 1949). In view of anticipated studies on relationship of this mosquito to transmission of certain poultry diseases, it was desirable to learn more about its habits. To facilitate the realization of this objective, some sort of apparatus was necessary. Thus, a rotary trap was designed and constructed for the purpose of studying host, color, light intensity and other preferences of this and other mosquitoes. Described in this report are the trap and the uses for which it was designed. Data obtained, particularly concerning *C. quinquefasciatus,* with the aid of the trap will be the subject of another paper.

DESCRIPTION OF THE TRAP. The trap proper is of sheet aluminum and from above appears hexagonal in shape, Figures 1 and 2.[2] It has six wedge-shaped compartments with partitions of solid aluminum riveted to the top and bottom. Toward the back of each compartment is a plate holder that will support filters to regulate color or intensity of light. The front side of each compartment has a door in which a screen inlet is mounted. The maximum opening of the rectangular inlet is 1.5 x 8 inches, the aperture of which can be reduced in size and changed in shape as desired. The depth of each inlet is 11 inches and behind each inlet, space

[1] The trap was designed by the authors and constructed by Ray Patterson of the Engineering Shops, Alabama Polytechnic Institute.

[2] Persons interested may obtain blueprints of the trap from the Department of Poultry Husbandry.

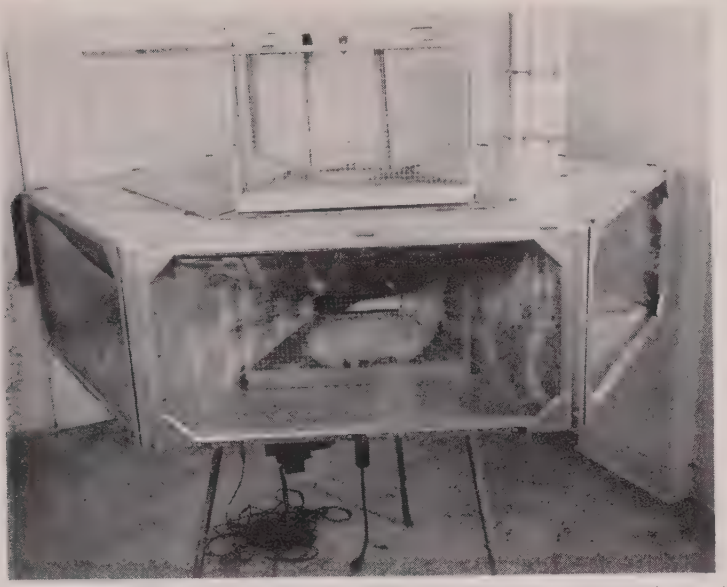

Fig. 1. Front view of one compartment of the six-compartment rotary inset preference trap with chicken in animal cage directly behind inlet cone. A sample animal cage is on top of trap.

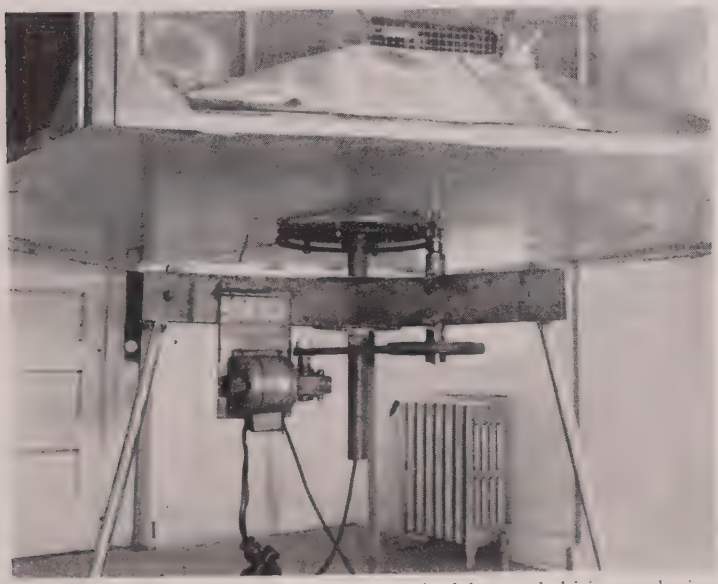

Fig. 2. View of underside of trap showing tripod base and driving mechanism

is available for a wire animal cage with dropping pan, test panels, delivery of CO_2 or other test subjects. There is also a top door to each compartment; thus the operator can introduce cages or other equipment by either route.

The trap proper is mounted on an adjustable tripod base, which is removable for ease in handling. The trap revolves around a central pole to which is mounted a stationary battery of six lights. The compartment, or turn-table portion, is electrically driven and the driving mechanism is designed to allow the trap to rotate at several speeds by merely changing pulley wheels.

Accessory items for the trap include glass cells and filter frames that can be placed in plate holders. Glass cells are water tight, designed to hold solutions of different colors and intensities. Designed, but not tried, are panels that can be painted with repellents or attrahents that can be placed behind the inlet. A CO_2 drum can be placed on the top of the trap with outlets to each of the compartments.

Prior to use, the trap was carefully tested to detect compartment differences. It was placed in the field under a canvas canopy near mosquito breeding sites and operated for several nights with white light, but without bait. Compartments rotated around the centrally illuminated pole at the rate of one complete revolution per 74 seconds. A comparison of the numbers of winged insects that entered each compartment revealed that there were no compartment differences. Collections included several species of mosquitoes as well as a good many other insects.

In a similar manner, the trap was operated for several nights in a heated, thermostatically controlled, mosquito-tight room. Mosquito breeding containers, which contained only stages of *Culex quinquefasciatus* were located in the room so that there was continuous emergence of adults during the test. Again, daily counts of male and female mosquitoes entering each compartment revealed that there was no difference between compartments.

In another series of nine trial runs, the trap was operated in the aforementioned room with Leghorn hens that had been carefully selected for similarity as baits. One hen was placed in each of three adjacent compartments for three successive nights. They were randomized to new compartments for each run. Since most of the mosquitoes that entered were females and they took blood meals, counts were based on mosquitoes that had fed. In spite of possible differences between similar hens in the different compartments, the difference between the numbers that fed on different birds was not significant. This was taken as another indication that there was no difference between compartments.

POSSIBLE USES. Of the several uses, the trap is suitable for making host-preference determinations. As many as six different animals can be placed in separate compartments or several smaller animals in each compartment and the attraction to and/or feeding on such animals by any winged, blood-sucking insect can be compared. Cage-size limits the size of animal that can be compared, and a 20-pound animal is about the limit of a single cage. The trap can be operated in the field or in a room where specific insects can be liberated. In addition, the attraction of insects to varied numbers of small animals or plants of different breeds or varieties, sexes, ages and sizes can be compared. The cages and compartments are easily cleaned between runs to eliminate odors.

By means of flat glass cells that hold colored dyes or by colored filters, the attraction or repellence of different colors of the spectra for winged-insects can be determined. By using dyes in cells rather than fixed filters, it is possible easily to alter the concentration of contents in order to study the effects of different light in-

tensities on insects. The attrahent effect of different concentrations of CO_2 for insects that feed on warm blooded animals also can be determined with this apparatus.

Data pertaining to some insects' attraction to or repellence by plants, animals, or other substances can be gained much more rapidly and less expensively by a trap of the design described herein than by some of the conventional methods of observation now being used. It is believed that a trap of this design may prove useful to other investigators interested in similar studies and that other uses for such a trap may be developed.

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RECONNAISSANCE OF MOUNTAIN MOSQUITOES IN THE McKINLEY PARK REGION, ALASKA

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Introduction. Since World War II, rapid progress has been made toward a checklist of Alaskan mosquitoes; yet knowledge of those of the alpine zone which comprise nearly a hundred thousand square miles is still meager and scattered. Published lists, general or local, for the forms actually breeding above timber line are lacking and needed. Distribution data based on collections of biting *Aedes* are considered unreliable, due to the uncertainties of identification and because the provenience of winged insects collected on mountainsides is problematic. It has proved difficult, if not impossible, to predict mosquito problems at high priority mountain installations. Reliable data are not only scanty but their interpretation is also complicated, since alpine conditions, habitats, and biota descend even to sea level under the influence of glaciers. Altitude itself, like latitude and longitude, may give no clue to local precipitation and terrain aspects including slope direction, exposure, valley width and air drainage, and local temperature inversions which greatly affect mountain climate. Fortunately, the distribution of trees may be used in Alaskan zoogeographical and ecological studies as a criterion indicating local weather, and woods *per se* are decisive in determining mosquito distribution. Thus it was shown by Frohne (1955a) for tundra mosquitoes that the same arctic tundra forms and but few others characterize subarctic tundra. Hence emphasis is placed on the timber line rather than on actual elevations in this mountain study. The present progress report of larval surveys in the McKinley Park region introduces investigations of Alaskan alpine mosquitoes.

In the most recent listing including Alaskan alpine mosquitoes, Jenkins (1948) reported six species of *Aedes* from elevations usually above timber line, viz.:

[1] Now at Schistosomiasis Control Pilot Project, Palo, Leyte, P. I.

Aedes communis, punctor, impiger (nearcticus), nigripes, intrudens, and *pullatus.* The first four are known Alaskan arctic species (Knight, 1951). Natvig (1948) considers the latter two to be arctic forms also in Scandinavia. Beyond this generalization that arctic forms do occur in the mountains, knowledge as to vertical range of the score of other Alaskan mosquitoes is very scanty. It was apparent in planning alpine studies that it would be particularly instructive to determine, as well, the occurrence of wigglers and their characteristic habitats at (1) the upper levels of spruce woods or about 2,000 feet, (2) the transitional terrain called taiga or the hemiarctic, which lies just below timber line, in addition to (3) the treeless alpine zone between timber line and perennial snow, which usually begins at 5,000 to 6,000 feet.

MOUNT MCKINLEY NATIONAL PARK. The Mount McKinley National Park situated between Anchorage and Fairbanks comprises about 3,000 square miles said to be 60 to 75 percent above the timber line. No publication on the Park mosquitoes antedated the present surveys. However, Wildlife Ranger H. E. Booth collected and reared mosquitoes for H. H. Stage of the Bureau of Entomology in 1945. Jenkins (l.c.) refers to trips to the Park by Alaska Insect Control personnel in 1947, and Park Service files reveal one party was led by C. S. Wilson, who sprayed three acres experimentally in April, another by Alan Stone, who checked the effectiveness of Wilson's spraying in June. It seems likely that Jenkins' apt designation of *Aedes pullatus* as a species of "alpine meadows above timber line" refers in part to Dr. Stone's dipping notes in McKinley Park on that occasion. Frohne (1955b) described swarms of *Aedes communis* and *impiger* males seen in the Park in 1954.

ACKNOWLEDGMENTS. Grateful thanks are due Superintendent Grant H. Pearson and his staff for counsel, hospitality, and logistic support, and to Dick and Gertrude Frohne for field and botanical assistance, respectively, and finally to Patsy Avery for making permanent mounts of representative larvae.

METHODS, AREAS, AND WEATHER. Representative larval collections were dipped along the Denali Highway connecting Park headquarters to (1) Wonder Lake about eighty miles to the west; and (2), in the opposite direction for about sixty miles from Park headquarters southward via Cantwell, then eastward to new road construction. Much of (2) is in the narrow Nenana valley. The 1954 collections were dated June 15, 16, 17, 24, 27 and July 3, 5, 7, 8. They came from elevations between 1,700 and 5,000 feet. The dipping in May 1955 included two road surveys of (2) and a foot survey of the phenologically early southeast slope of Mount Healy. Collections were made as weather permitted May 10–25 from elevations of about 1,700 to 3,000 feet.

Identification of mounted *Aedes* larvae was materially aided by saddle spine characters (Frohne, 1955c). Apical spine traits of fourth instar larvae are consistently recognizable as early as the second instar and in favorably mounted specimens usually so in the first instar.

Small larvae of *Aedes intrudens* and *nigripes* proved puzzling. The saddle spines of these species had not been studied.

When larger larvae were later collected in the same habitats where doubtful *intrudens* or *nigripes* small larvae had been taken, it was evident that the earlier collections of first instar larvae belonged to *nigripes.*

RESULTS. When the data are classified according to the vertical distribution of the species in the 3 major mountain zones (*woods,* below 2,000 feet; *taiga,* 2,000 to 3,000 feet; and *alpine,* above 3,000 feet), the following generalization can be made. *Aedes communis* (DeG.) was the most abundant and also the earliest species below about 3,000 feet. *A. impiger* (Wlk.) and *pullatus* (Coq.) also occurred abundantly in all three zones, the former appearing considerably earlier. *A. excrucians*

(Wlk.), which hatched about the same time as *pullatus,* was common to abundant in the two lower zones, not uncommon but about a month later above 3,000 feet. *A. punctor* (Kby.) and *hexodontus* (Dyar) were common in bogs only, but both occurred at all three levels. The remaining larvae were apparently restricted to lower, wooded zones, but the data are too few for conclusions. Two species of *Culiseta, alaskaensis* (Ludl.) and *impatiens* (Wlk.) were common on the wing at times in May and June. However, only thirteen larval collections of *Culiseta* were made, composed mostly of small larvae I could not identify with certainty. All were collected below 3,000 feet. Certain Central Alaskan mosquitoes, common elsewhere at low elevations, were *not* found in these mountain surveys, e.g., *Culex territans* (Wlk.), *Anopheles* sp., *Culiseta morsitans* (Theob.), *Aedes implicatus* (Vock.), *stimulans* (Wlk.), *flavescens* (Müller), and *decticus* H., D., and K. (*pseudodiantaeus* Smith).

Larval Habitats. The following outline-key to eleven major larval habitat types provides only a framework for organizing the ecological data, but it should be useful with certain reservations as a general guide for later work. To save space and simplify the classification several omissions were made such as disregarding transitional habitats between types, and the temporary margins of permanent type waters including all microhabitats. As a result some strange bedfellows have been lumped as they occasionally seem to be in Nature. The key word, designated by capitals, facilitates discussion, and the Roman numerals designate types in Table 1.

Outline-Key to Larval Habitat Types. Pools and ponds below timber line protected by marginal trees and shrubs.

Bog waters, dystrophic, in peaty depressions, choked with vegetation.

Deeper, permanent bog pools.

Type I. Sphagnum-*Rubus-Eriophorum* community.... 7 examples

Type II. Pinguicula-*Carex* community ..... 5 examples

Type III. Ledum and/or *Betula nana* communities..12 examples

Swamps and marshes, eutrophic-oligotrophic, rarely dystrophic, shaded or weedy, temporary or semipermanent, though often with permanent depressions.

Shallow, temporary vernal waters, usually within scrubby woods.

Type IV. Marginal trees of Salix-*Picea* community.. 18 examples

Type V. Barren and exposed, or grassy Taiga pools and ponds... 18 examples

Type VI. Ranunculus Flammula flats ..... 2 examples

TABLE 1.—Number of individual habitats belonging to eleven types in which each larval species was taken

Types	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
A. communis	3	1	6	13	15	2	4	2	4	3	
A. impiger		1		6	11	1	1		1	3	
A. pullatus	1	3	3	6	2	1	5	4	24	6	
A. punctor	5	4	1		2	1	7	1			
A. pionips	2	2	1	4	1		1			1	
A. excrucians		2		1	1	1	5				
A. hexodontus	3	1	1		1		3				
C. alaskaensis				1			6			1	
A. intrudens				1	2		2				
A. cataphylla				1	2	1	1				
C. impatiens	1			1			3				1
A. nigripes					1	1	1				
A. cinereus	2										
A. fitchii							1				
A. diantaeus		1									

Deeper, semipermanent or permanent, weedy ponds.

Type VII. CAREX communities.... 20 examples

Exposed pools above or below timber line, virtually unprotected by marginal plants, barren of or scantily occupied by vascular aquatic vegetation.

Natural (long-standing) depressions, water permanent or temporary.

Type VIII. Cold ALPINE pools fed by melt of adjacent snow-ice fields, *Caltha* community ..... 4 examples

Type IX. Warm, intermittent summer pools in dry beds of TORRENTS, gravelly flood plains, barren or with sprigs of *Equisetum, Carex,* or *Juncus*.. 24 examples

Man-made (recent) depressions, usually temporary.

Type X. Barren BORROW pits, ditches, caterpillar tracks, etc.... 9 examples

Type XI. Artificial CONTAINERS (wash tub) ..... 1 example

Particular larval species more or less satisfactorily characterize each proposed habitat type arrived at without consideration of dominant larva or larvae present. The special advantage of these eleven habitat types chosen is that practically any pool may be classified almost at a glance and notes limited to peculiarities. Table 1 gives the distribution of the fifteen larvae in the eleven types indicated by the number of habitats positive for each. The table presents numbers of positive habitats, not individual collections. Nor is it justifiable from these preliminary qualitative dipping surveys to quantify by citing actual larval counts. However, it is perhaps descriptive of habitat types to note the dominant species as the characteristic larva. *A. punctor* and *hexodontus* were the typical larvae of SPHAGNUM bog pools (I), and *punctor* was taken in four out of five PINGUICULA pools (II), as well. LEDUM bog waters (III), usually more transient and formed early, favored *A. communis,* which is also the characteristic larva of SALIX pools (IV). Associated *A. impiger* and *communis* teemed in most TAIGA pools (V). In the peculiar RANUNCULUS FLAMMULA flats (VI) the comparative scarcity of both *communis* and *impiger* was striking, and one also received the impression that the collection of *A. nigripes* in numbers here, and here only, was ecologically significant. The chief reasons for the miscellaneousness of the larvae taken in CAREX ponds (VII) seem to be the confluence of temporary, vernal, marginal water early in the season with this permanent type, and the variability in elevation, water source, temperature and color. However, by late June CAREX ponds, even of different elevations, were more uniform and *A. excrucians* and *punctor* with the *Culiseta* larvae were characteristic. *A. pullatus* was the typical wiggler of ALPINE pools (VIII), and equally so of TORRENT pools (IX) in all three vertical zones. For both these habitat types occurring within the trees, however, *A. communis* breeding preceded *pullatus,* especially in shallow, sunny gravelly flats. *A. pullatus* also frequented new and artificial habitats like BORROW pits more than any other species. However, only *Culiseta impatiens* bred in an artificial CONTAINER habitat (XI), an intermittently used wash tub.

LARVAL ASSOCIATION. Larval association is concerned primarily with different larval species of the same habitat and may also take prevalences and phenologies into account. To be "associated," northern *Aedes* would ideally leave the egg the same day, and also pupate more or less simultaneously. No two Alaskan species are so closely matched. On the other hand, in emphasizing the common habitat it is often useful to regard all larval species occurring in a particular pool during the entire season as associated. The method of determining association for Table 2 was a compromise. Association indicates occurrence in the same dipping collection, thus stressing similar seasonal history and soft-pedaling habitat. For example, *A.*

TABLE 2.—Associations of McKinley region mountain larvae. The diagonal figures are the total collections of each species

	communis	*impiger*	*pullatus*	*punctor*	*pionips*	*excrucians*	*hexodontus*	*alaskaensis*	*intrudens*	*cataphylla*	*impatiens*	*nigripes*	*cinereus*	*fitchii*	*diantaeus*
communis	73														
impiger	26	34													
pullatus	11	9	52												
punctor	8	1	8	19											
pionips	6		8	5	14										
excrucians	3	3	3	1	1	10									
hexodontus	6		1	6	2		9								
alaskaensis			2	1				8							
intrudens	4	2				1			5						
cataphylla	5	7	2	1		1			1	7					
impatiens			1	1		1	1	2			5				
nigripes	3	4	2	4		1				2		5			
cinereus	1			2	1		2				1		2		
fitchii						1								1	
diantaeus			1	1	1										1

communis and *pullatus,* the most common larvae, were associated in only eleven of 125 *communis* plus *pullatus* collections, an index of association under 10 percent. This index would be much higher, however, if association were determined by occurrence in the same breeding place and the different collections lumped, because May *communis* larvae in persistent TORRENT pools, etc., are usually replaced in June and July by *pullatus* larvae. In the present reconnaissance, however, no distinction was made between large and small larvae. As a result the later (small) larvae of one species associated with earlier (large) larvae of a second species could not have hatched or grown in the ice-cold water which nurtured the earlier species. About 24 percent of *impiger* plus *communis* collections contained both species, which are early, common, and in one habitat type, the TAIGA (V), even codominants. The other pairs of larvae collected enough times so that their percent of association may be significant were: *punctor* and *hexodontus,* over 20 percent; and *punctor* and *pionips,* about 15 percent. It appears likely, since larval samples were small, that some association percentages, like 17 percent for *cataphylla* and *nigripes,* larvae which occurred "diluted" in *communis* collections, are too low.

SEASONAL OCCURRENCE. The phenology data for larvae found in the Park area will assist the planning of later workers, but the dates probably vary by as much as ten days for different years. Below timber line in 1955, when spring was late, the larvae of all thirteen *Aedes* were collected the latter half of May. None of the earliest species, *communis,* hatched in April, although this may be the case in some years. June and July collecting in 1954, a normal season, indicated that above the timber line these months correspond to May for the six *Aedes* larvae which were taken at all three vertical zones. Making exceptions for delayed hatching of eggs in the larger, later melting habitats, the succession of species below timber line by groups of more or less contemporaneous species was: (1) *A. communis;* (2) *A. impiger, punctor, hexodontus, cataphylla, nigripes, intrudens;* (3) *A. pullatus, pionips, excrucians, diantaeus, fitchii;* (4) *C. alaskaensis* and *impatiens.* The *A. cinereus* larvae were taken May 19 and July 5, but the species is believed to belong in group (3).

The order of occurrence differs from Jenkins (l.c.), mostly, I think, because he combined collection data of different latitudes and altitudes.

SUMMARY. Preliminary dipping surveys of mosquito larvae were made in the mountainous McKinley Park region of Alaska from which fifteen mosquitoes were identified: *Aedes communis, impiger, pullatus, punctor, hexodontus, excrucians, pionips, intrudens, cataphylla, nigripes, cinereus, fitchii, diantaeus, Culiseta impatiens,* and *alaskaensis.*

The vertical distribution of the larvae was related to three mountain zones dependent on altitude and presence of trees: (1) below 2,000 feet (woods); (2) between 2,000 and 3,000 feet (taiga); (3) above 3,000 feet (alpine). The first six mosquitoes listed occurred in all three zones.

Eleven principal types of larval habitats were recognized on the basis of vegetation, water, depression, and elevation where more or less characteristic assemblages of larvae bred in an orderly seasonal succession which was later at successively higher elevations.

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NOTES ON THE DISTRIBUTION OF MOSQUITOES IN MARYLAND AND VIRGINIA [1]

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Marshall (1938) stated that every "locality record" is the result of a fortuitous meeting between a collector and his quarry. A few fortuitous meetings are reported here. Some of them were preceded by a rather diligent search.

Psorophora (Janthinosoma) ferox (Humboldt) under the name of *Janthinosoma musica* Say was reported as being "not rare in Maryland" by Symons, Coffin, and Gahan (1906). This reference was apparently overlooked by Carpenter and LaCasse (1955). *P. ferox* probably occurs throughout Maryland, but no collections have been recorded for the western counties. Rajapaksa (1956) found that females were attracted to skunks, raccoons, opossums, rabbits, squirrels, and woodchucks at the Patuxent Research Refuge near Laurel. Larvae have been found on rare

[1] Miscellaneous Publication No. 276, Contribution No. 2748 of the Maryland Agricultural Experiment Station, Department of Entomology.

occasions in the College Park area in August.

Psorophora (J.) horrida (Dyar and Knab) was reported from Woodstock, Virginia, by Dorer *et al.* (1944) and by Roth (1945); a single specimen (Dyar's determination) is in the U. S. National Museum. Roth (1945) also reported that among Dyar and Knab's material is a specimen of *P. horrida* from Plummer's Island, Maryland. These two specimens along with 4 females from the District of Columbia (Good, 1945) were collected from 1904 to 1909, and it appears that no additional specimens have been taken during the intervening years. Consequently, it is of some interest to report the occurrence of *P. horrida* at the Patuxent Research Refuge near Laurel, Maryland. Three females were taken while attempting to bite on August 27, 1955, by J. T. Whitlaw, Jr., and several specimens were collected by B. R. Evans in August 1956 in the same area.

Psorophora (J.) varipes (Coquillett). Three females labeled Pocomoke Swamp, October 13 or 16, 1936, were found in the University of Maryland Collection and identified by the author. Subsequently Dr. Walter A. Connell pointed out that these specimens had been collected by him near Libertytown, Worcester County, and also called attention to his paper (Connell, 1941) in which *P. varipes* was recorded from Maryland. This paper has been overlooked by several authors. Two other specimens taken by Connell in 1936 are in the University of Delaware Collection; one of these bears the determination label of G. H. Bradley. Changes in the swamp channels during the last twenty years make it doubtful if *P. varipes* still breeds in the area.

Psorophora (Grabhamia) discolor (Coquillett) was recorded from Grassymead, Virginia, by Dyar (1922), but this record was overlooked by Dorer *et al.* (1944). This species is rare in Maryland. The only record is apparently that of Cory *et al.* (1934) from LaPlata.

Aedes (Ochlerotatus) aurifer (Coquillett) was collected at Cambridge, Maryland, in 1933 (Cory *et al.*, 1934). Four females from Cambridge, collected May 22, 1945 are in the U. S. National Museum. Eight females were collected in June 1937 in the Pocomoke Swamp and deposited in the University of Maryland Collection. George B. Vogt also collected this species at Indian Head, Charles County, in June 1945.

Aedes (O.) fitchii (Felt and Young) was collected by J. T. Whitlaw, Jr., on July 6, 1955, at the Patuxent Research Refuge near Laurel, Maryland, a new record for the state. The single female was determined by Dr. Alan Stone. It is hoped that additional material can be obtained.

Aedes (O.) grossbecki (Dyar and Knab) was recorded from Baltimore, Maryland, and Grassymead, Virginia, by Dyar (1922), but the latter record was overlooked by Dorer *et al.* (1944).

In the University of Maryland Collection are 4 females collected by T. H. Coffin near Easton, Talbot County, Maryland, in June 1906. George B. Vogt made the determination subsequently. Dr. Dale W. Jenkins (personal communication) found that this species was fairly abundant at Edgewood, Maryland, in 1945. A specimen collected on April 1, 1945, is in the U. S. National Museum. Major changes in local habitats have apparently prevented breeding in this area in more recent years.

Aedes (O.) infirmatus (Dyar and Knab) was recorded by Connell (1941) from the Pocomoke Swamp near Libertytown, Worcester County, Maryland. Identification was confirmed by Dr. Stone, and two specimens are presently in the University of Delaware Collection. These were taken on September 23 and October 7, 1939. As mentioned above the paper by Connell (1941) has been overlooked by various authors.

Aedes (O.) punctor (Kirby) was reported by Bishopp *et al.* (1933) from Cambridge and Berlin, Maryland, and by Cory *et al.* (1934) from Chestertown

and Salisbury. Dr. Alan Stone discussed these records with the writer and is now of the opinion that all records of this species from Maryland are questionable. No specimens of *A. punctor* on which the published records could have been based can be found in the Museum. At least one specimen from the original material has been subsequently determined as *A. canadensis* (Theobald) by examination of the male terminalia. Darsie *et al.* (1951) reported that *A. punctor* has not been found in New Jersey, southern Pennsylvania, Delaware or Maryland.

Aedes (O.) sticticus (Meigen) was recorded from Woodstock, Virginia, and Herzog's Island, Maryland, by Dyar (1922). In the U. S. National Museum there is also one of Dyar's specimens from Cabin John, Maryland. Herzog's Island is in the Potomac near Cabin John (both Montgomery County). A single female was collected at the Patuxent Research Refuge near Laurel on August 30, 1956, by B. R. Evans. Confirmation of determination was made by Dr. Stone.

Aedes (O). stimulans (Walker) was collected by C. W. McComb, at Easton, Maryland (1 female), and at Gloucester Point, Virginia (2 females), in June 1956. The range of this species along the East Coast is thus extended southward from Delaware.

Aedes (O.) thibaulti (Dyar and Knab). A few females of this species were collected by the writer in the Seashore State Park, Cape Henry, Virginia, during May and June, 1945. This was thought to be the northernmost record in the eastern United States, but Dr. Stone found in the U. S. National Museum two females collected by Newhouse in Ellendale Swamp, Delaware. This is a previously unpublished record for Delaware.

Aedes (Finlaya) atropalpus was omitted from the list of Dorer *et al.* (1944). It had been reported from Virginia by Dyar (1922). Larvae of this species are abundant in rock holes along the James River at Richmond and on the Virginia and Maryland sides of the Potomac above Washington, D. C.

The following species have been recorded from Virginia but not from Maryland: *Orthopodomyia alba* (Baker)—also District of Columbia (Good, 1944) and New Jersey—*Psorophora (J.) cyanescens* (Coquillett), *Aedes (O.) dupreei* (Coquillett), *Aedes (O.) thibaulti* (Dyar and Knab, and *Culex (Melanoconion) peccator* (Dyar and Knab)—also from Delaware and the District of Columbia.

The following species have been recorded from Maryland but not from Virginia: *Aedes (O.) aurifer* (Coquillett), *Aedes (O.) fitchii* (Felt and Young), *Aedes (O.) infirmatus* (Dyar and Knab), and *Aedes (Aedes) cinereus* (Meigen).

In this paper the following species are reported for the first time from Maryland: *Aedes (O.) fitchii* (F. and Y.), and *Aedes (O.) stimulans* (Walker); and from Virginia: *Aedes (O.) stimulans* (Walker) and *Aedes (O.) thibaulti* (D. and K.). Previously published records of *Aedes (O.) punctor* (Kirby) are questionable.

ACKNOWLEDGMENTS. The preparation of these notes would have been impossible without the generous help of Dr. Alan Stone, Entomology Research Branch, U. S. Department of Agriculture. He has determined or confirmed determinations of many of the specimens mentioned. Messrs. J. T. Whitlaw, Jr., B. R. Evans, and C. W. McComb, graduate students at the University of Maryland, have given permission for their collection records to be included, and grateful acknowledgment is made to them. To Dr. W. A. Connell of the University of Delaware special thanks are extended for calling attention to his paper in *Mosquito News* when this journal was in its infancy and for supplying additional information about his collections made in 1936 and 1939. Dr. D. W. Jenkins, Mr. G. B. Vogt, Mr. R. E. Dorer, Dr. R. F. Darsie, Jr., and Mr. Donald MacCreary also contributed to this report.

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A SIMPLIFIED METHOD FOR LARGE-SCALE LABORATORY REARING OF *AEDES AEGYPTI* (L.) [1]

J. A. McKIEL [2]

Large numbers of mosquitoes are frequently required for such laboratory studies as basic physiological reactions, insecticidal resistance, and evaluation of insecticides and repellents. At our laboratory, large numbers of *Aedes aegypti* (L.) have been used in the preparation of extracts for use in studying reactions to mosquito bites. The rearing of mosquitoes for this purpose has required, in the past, many hours of tedious effort at the expense of the main problem under investigation. Recently, however, a method for mass-rearing *A. aegypti* has been devised, with the result that the time spent in rearing has been reduced to a fraction of that required previously.

Methods in general use for rearing *A. aegypti* under laboratory conditions are fairly standard, and, except for minor variations, follow the procedures described by Granett and Haynes (1944), and Trembley (1944, 1955). Large-scale rearing by these procedures involves the use of a large number of trays. The handling of so many trays each day requires careful manipulation, and is, therefore, time-consuming. The separation of pupae from larvae is likewise a tedious and slow process.

Description of Rearing Unit. In an attempt to reduce the time spent in rearing, a unit was constructed in such a way that large numbers of larvae could be reared to adulthood without separating pupae from larvae while at the same time eleminating the use of a large number of trays. Figure 1 is a photograph of the unit, and Figure 2 is a sectional view. The tank was 30 in. long, 14 in. wide, and 36 in. high, and was made of galvanized iron. The top section was tapered, beginning 24 in. from the bottom, to form an opening 6 in. square. Three

[1] Contribution from the Department of Biology, Queen's University, Kingston, Ontario.

[2] Laboratory of Hygiene, Ottawa, Ontario.

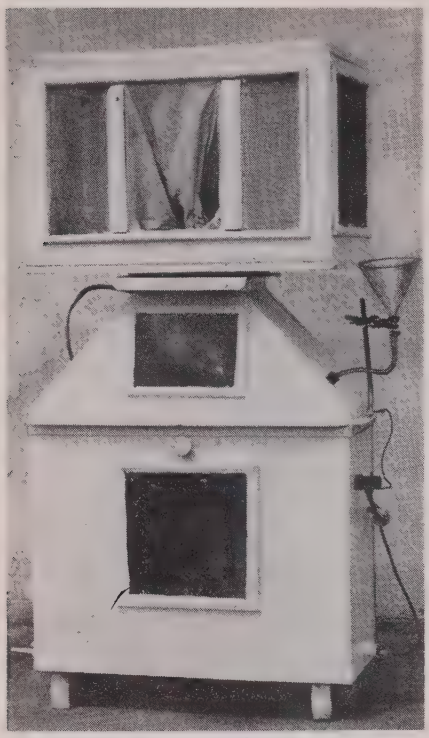

FIG. 1. Photograph of rearing unit.

pieces of metal screening (f) were soldered within the tapered portion to provide resting places for newly emerged adults. A slide (d) was fitted across the opening at the top of the tank. Sponge-rubber stripping was cemented to the top edge of the tank to form a seal between the tank and a removable cage (a).

The cage measured 30 in. long, 20 in. wide, and 20 in. high, and had in its floor a centrally located opening of 5 in. diameter. The opening could be closed by a swing-type cover (c). During the time that the cage was being stocked with mosquitoes, a removable metal-screen funnel (b) was placed over the opening to deter mosquitoes from re-entering the tank.

Tap water was added to the tank to the 24-inch level (approximately 165 l.). A copper air-line (h) was inserted, and its position was adjusted to about 4 in. below the surface of the water. The air-line had three orifices, .015, .078 and .094 in., the largest being near the closed end of the copper tube. Aeration at 600 ml. per hr. was used to inhibit film formation on the surface of the rearing medium. The amount of surface disturbance was regulated by minor adjustments in the position of the air-line and in the rate of aeration. The room was maintained at 26° to 28° C. The rearing medium was held at 28° ± 0.1° C. by means of a 1000-watt heater (j) and a thermoregulator (g) built into the tank. A funnel (e) was used to add food and larvae to the rearing medium. A tap (k) enabled the tank to be drained as necessary. Two windows were provided in the front of the tank to permit inspection of conditions inside the tank (see Fig. 1). Normally these windows were kept covered with black cardboard.

REARING METHOD. Filter papers on which *Aedes aegypti* eggs had been deposited were immersed in water containing a few drops of yeast suspension and left overnight for the eggs to hatch. The following day the papers were removed, the number of larvae was estimated, and the water containing the larvae was poured into the tank. Estimates were made by counting 1,000 larvae into a minimum amount of water in a test tube and using this tube as a standard for making up additional lots of 1,000 larvae. Dog biscuits and Fleischmann's dry yeast were soaked in water and added to the contents of the tank. The feeding schedule was as follows:

Days after Hatch	Dog biscuits (mg. per larva)	Yeast (mg. per larva)
1	1	0.2
5	2	0.2
7	2	--

Pupation began on the sixth day after hatching. Emergence of adults began on the eight day, reached a peak on the tenth to twelfth day, and was 99 percent complete on the sixteenth day. When the cage

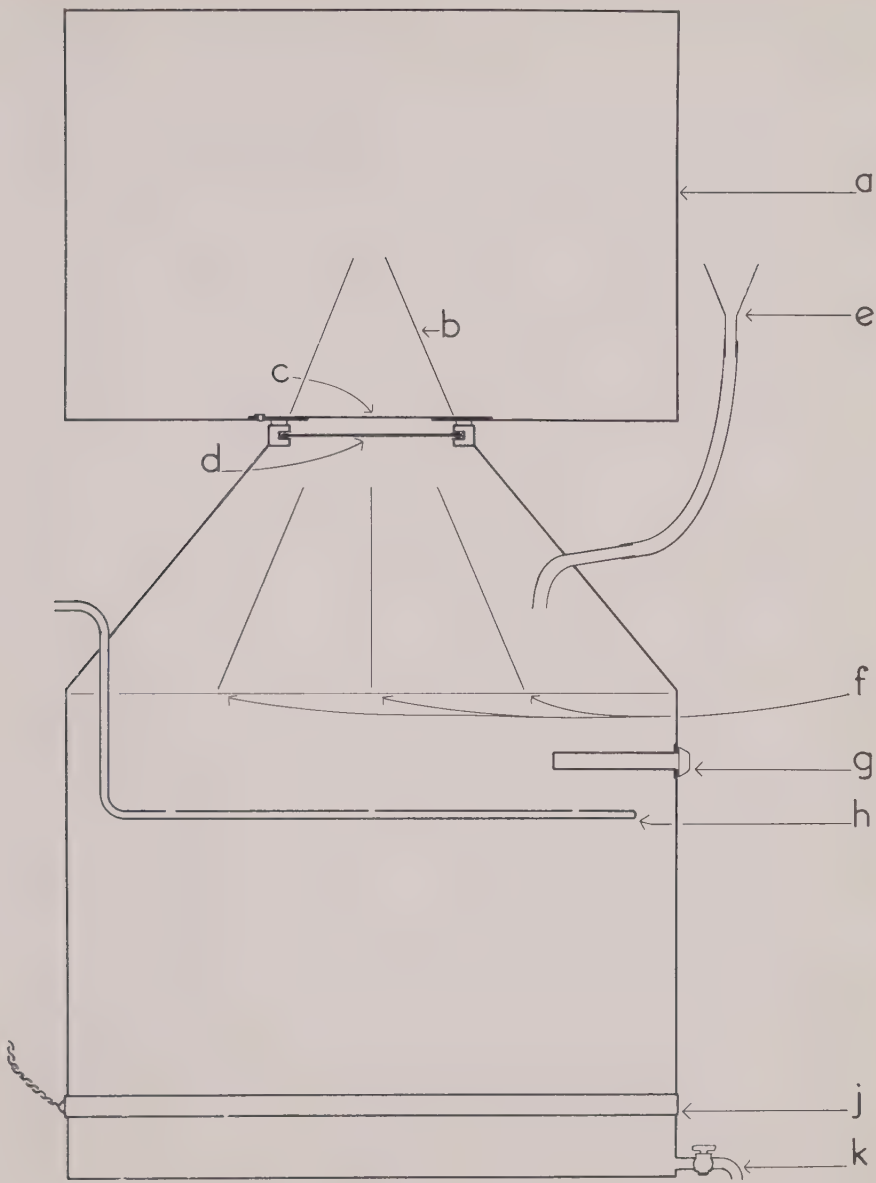

Fig. 2. Diagrammatic sectional view of rearing unit.

was fully stocked (about 4,000 mosquitoes as based on past experience with this size of cage), the slides (c) and (d) were closed, the screen funnel (b) was removed, and the full cage was replaced by an empty one. The slides were opened, the screen funnel was inserted, and the new cage was ready to be stocked.

Several trials were made with three rearing units to arrive at the feeding schedule described above and to obtain an approximation of the maximum number of larvae that could be reared satisfactorily in one unit. As a result of these trials, it was found that 40,000 larvae per unit (about 4 larvae per ml.) could be reared at one time, and that this number produced large, healthy-appearing adults whose longevity did not differ noticeably from those reared by the tray method. Larger numbers than this usually resulted in undersized adults and in prolongation of the aquatic stages. The addition of amounts of food larger than those shown in the feeding schedule frequently was followed by the formation of a surface film in the corners of the tank. This suggests that a circular rather than a rectangular tank might be preferable. The dimensions of the tank were arbitrarily chosen, and no attempt was made to determine an optimum surface-to-volume ratio of the rearing medium. Although considerable numbers of larvae were found to be feeding at the bottom of the tank at any given time, this does not preclude the possibility that a larger number of larvae per ml. could be reared satisfactorily by altering the surface-to-volume ratio, thus effecting a saving in the space requirements. Examination of the sediment in the tank at the end of satisfactory runs indicated that the mortality of the aquatic stages was negligible. Also, counts made of the adults obtained from such runs were within 20 percent of the estimated number of larvae added to the tanks. The rate of production by the above method is, therefore, approximately 2,500 adult mosquitoes per rearing unit per day. The time required to maintain this rate of production in three units is about two hours per week per unit. Although these units have been used to rear only *A. aegypti,* it seems likely that the method could also be used to rear *A. atropalpus* (Coq.) and probably some of the other mosquito species.

After each of the runs referred to above, the three rearing tanks were drained and washed out thoroughly. To determine whether it is necessary to discard the rearing medium after each run, a fourth unit was kept continuously stocked with *A. aegypti* larvae. The population of larvae in the tank varied from time to time, but never fell below 5,000. The periodic requirements for additions of yeast and dog food were estimated by visual inspection of the contents of the tank. This unit was operated continuously for eight months, and at no time was there any evidence that products toxic to the larvae had accumulated. The tendency towards film formation from addition of excess food appeared to be much less than in a tank recently placed in operation. On the few occasions that a film began to form on the medium, the film was removed with a bottle brush, and about one-quarter of the medium was drawn off and replaced with tap water. This method was found to be adequate for preventing further film formation.

The fact that the same larval medium can be used continuously means that a great saving of distilled or treated water is possible in areas where tap water is not suitable for rearing mosquitoes. The continuous method is especially useful during periods when the colony is merely being maintained. For maximum production, however, a chemical method for determining the amount of food to be added periodically to the medium would be desirable.

SUMMARY. A method is described for rearing large numbers of *Aedes aegypti* with little effort and attention. The rearing unit consists of a collecting cage

mounted on top of a thermoregulated tank. Aeration is used to inhibit film formation on the surface of the rearing medium. When the collecting cage becomes filled with mosquitoes, it is easily replaced by an empty cage. One of these units has been used to rear *A. aegypti* continuously for eight months with no signs of accumulating toxic products in the rearing medium.

Acknowledgment. The author wishes to thank Dr. A. S. West, Professor of Zoology, Queen's University for his helpful suggestions.

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WINTER BREEDING OF *CULEX QUINQUEFASCIATUS* SAY IN COLUMBUS, GEORGIA

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During the past decade we have noted the occurrence from time to time of varying numbers of *Culex quinquefasciatus* Say larvae and adults throughout the winter months at different localities in the State of Georgia. These more or less random observations established the fact that this species overwinters in this latitude in both the larval and adult stages, and the persistence of very small larvae, even during the coldest months, suggested that reproductive activity might continue throughout the winter.

The literature contains many references to the overwintering habits of anopheline species, but fewer on the corresponding activities of the southern house mosquito. Dyar, 1922, and Matheson, 1944, describe *Culex quinquefasciatus* as a species that survives the winter in the more northern sections of the country as gravid, hibernating females. Dozier, 1936, Carpenter, 1941 and Shlaifer and Harding, 1946, observed larvae of this species during the winter season in Louisiana, Arkansas, and Tennessee, respectively, but did not record finding egg rafts or other data indicative of reproductive activities. Hinman, 1931, found breeding and biting activities of *Culex quinquefasciatus* continuing through the winter at New Orleans, and Horsfall, 1937, observed egg rafts of this species as late as December 3 in Arkansas.

So far as we have been able to ascertain, the overwintering habits of this mosquito in Georgia have not been adequately studied, and this paucity of information on its bionomics in a latitude where climatic conditions differ considerably from those prevailing in the areas investigated by the workers previously cited appeared to warrant a more thorough investigation of the behavior of *Culex quinquefasciatus* in Georgia during the colder months.

Several considerations prompted the selection of the city of Columbus, Georgia (Latitude 32°, 31′ N.) as the principal locality for the study. The city-county

health department is keenly interested in mosquito control, and encouraged the investigation by supplying personnel to assist in collecting data. In addition, Columbus enjoys the somewhat dubious advantage of a wealth of permanent and semi-permanent aquatic situations which are ideal for the propagation of *Culex quinquefasciatus*. The city contains approximately four thousand street catch basins located on combination storm and sanitary sewers, and a system of surface drains, practically all of which hold water the year round. These mosquito breeding areas are supplemented by many other and diverse situations of a less permanent nature mostly in the form of water-holding domestic rubbish which may be found littering private premises in the less favored portions of this and many other cities. These numerous breeding habitats produce swarms of domestic mosquitoes, which in addition to providing ample material for study, necessitate that the health department carry on a control program during the warmer months for their suppression.

The collection of data for this study began on October 18, 1955, somewhat in advance of the official beginning of winter and continued through March 20, 1956. The months of October, November, December and January were unusually cold and dry with temperatures ranging from one to eighteen degrees below normal for the greater portion of this period. The preceding summer and autumn also had been exceptionally dry, and this protracted drought prevented the accumulation of water in artificial receptacles on private premises and in some ground pools and ditches. Consequently, from October until February *Culex* breeding was more or less restricted to the more permanent situations, and principally to the street catch basins. Weekly collections demonstrated the presence of *Culex quinquefasciatus* egg rafts, larvae, and adult mosquitoes in these situations throughout the greater part of the winter. Viable egg rafts were collected from the catch basins in every month from October through January. Twenty-four egg rafts were found on December 28 following a week in which the mean minimum temperature was 45° F. A maximum number of eight egg rafts was found in a single catch basin on that day. It seems worthy of note that several egg rafts were collected on January 5 and again on January 18 following periods of 5 and 9 days, respectively, when the daily minimum temperature was near to or below the freezing point. Both larvae and adult mosquitoes were particularly numerous in the catch basins. Random dips in these environments with an ordinary pint capacity enameled dipper frequently produced from 100 to 300 larvae of all stages per dip. These numbers were actual counts and not estimates. The interior walls of the catch basins appeared to be the favored diurnal resting places for adult *Culex*. They were difficult to capture in these shelters, but were easily dislodged and forced to fly out by raking over the interior walls with a bamboo pole. The numbers thus observed were surprisingly large and included both male and female mosquitoes. Even during the coldest weather, estimates of the total adult mosquitoes dislodged from individual catch basins were frequently as high as one hundred or more. The aggregate numbers of adult mosquitoes finding refuge in these protected shelters during the winter must be tremendous, and are very likely responsible for the majority of the complaints of biting activity which are discussed later. In Georgia, the males of this species appear to be more hardy than their homologues further north which are said by Matheson (1944) to die at the advent of cold weather. Male *Culex* in Columbus were sometimes observed in active flight when air temperatures were at the freezing point.

From October through January very few adult mosquitoes were found in diurnal shelters other than the catch basins. This probably resulted from a combination of the following circumstances. First, continued dry weather had

all but abolished the domestic production of mosquitoes on private premises which undoubtedly depleted the population of adults immediately adjacent to these shelters. Second, from 1946 through 1951 all outbuildings and similar daytime resting places for adult *Culex quinquefasciatus* in Columbus were given seasonal treatments with residual DDT sprays. It is possible that enough of the chemical remains on the interior walls of these shelters to discourage mosquitoes from resting on the treated surfaces. Third, the temperatures and humidity prevailing in the catch basins probably provide a micro-climate more favorable for the survival of mosquitoes than other less protected shelters offer. It was not feasible to measure air temperature inside the catch basins; however, our records show that daytime water temperatures in the basins did not go below 52° F. This is several degrees warmer than the temperatures attained in unprotected surface waters, and it is probable that the air temperatures in the basins were consistently higher than those recorded outside.

Precipitation, in the form of rain, which began on the first of February and continued intermittently throughout the month, flushed out the street catch basins and surface drains so effectively that neither egg rafts nor larvae could be found in these situations for the greater part of the month. At the same time, however, the precipitation filled wash pots, metal drums, old auto tires and similar water-holding debris on private premises in the city and after the first week in February, egg rafts and larvae began to appear in these containers. Air temperatures fluctuated during February and March from a minimum of 27° F. on February 29 to a maximum of 82° F. on March 7. By the middle of March *Culex* breeding had become fairly common throughout the city, and since spring had arrived in this portion of Georgia by March 20, our investigations were terminated on that date.

These studies were supplemented by observations made during the winter months in other parts of Georgia. On January 9, hundreds of adult male and female *Culex quinquefasciatus* were observed resting beneath a bridge on Ocapilco Creek, a sluggish, sewage-polluted stream at Moultrie, Georgia. One egg raft and numerous larvae and pupae of this species were collected from quiet spots along the edge of the stream. On January 16, enormous numbers of *Culex* larvae in all stages of development were found in another sewage-polluted stream at Adel, Georgia. One egg raft was collected and numerous adult *Culex quinquefasciatus* were dislodged from beneath vegetation and stone piles. On March 9, large numbers of larvae of this species were discovered in ground pools at Augusta, Georgia, and many adult mosquitoes were seen resting on the overhanging banks of the pools. Numerous complaints about the biting activities of mosquitoes were received throughout the winter by the health department in Columbus, and on one occasion late in December, complaints became so vociferous that it was deemed necessary to larvicide the street catch basins in that section of the city. At Baxley, Georgia, similar complaints were received with such frequency during the colder months that it was necessary to continue routine larviciding operations throughout the entire winter.

The results of these studies demonstrate that from approximately the fall line southwards, *Culex qiinquefasciatus* is able to carry on reproductive activities throughout the entire winter in Georgia, and that even in abnormally cold weather, it maintains itself in sufficient numbers to create occasional problems of mosquito control. It seems reasonable to assume that the reproduction and survival of considerable numbers throughout the winter results in a rapid buildup of mosquito populations early in the spring and summer. This might be prevented by initiating control operations late in the winter.

The nuisance value of *Culex quinquefasciatus* is sufficiently great to place it in

the category of a major pest regardless of any relationship to human health and welfare. There are an almost infinite number of breeding places available for this mosquito in the urban areas of the state, and the total number of persons affected annually by the attacks of this insect must be counted in the thousands. In addition to the painful bites inflicted on its victims, this mosquito is known to serve as the vector of human filariasis and also as the vector of several diseases of domestic animals. It also seems worthy of note that the virus of western equine encephalitis was recently isolated from this species in North Carolina. R. W. Chamberlain (personal communication) has found that *Culex quinquefasciatus* is refractory to infection by this organism. He is of the opinion that the isolation resulted from the presence of the virus in bird blood which had been freshly ingested by the mosquito, and that it does not necessarily mean that this mosquito would be able to transmit the disease. The isolation of the virus from this species, however, is interesting and not entirely without significance.

We gratefully acknowledge the assistance of Mr. Dixon R. Olive, Jr. of the Muscogee County, Georgia, Health Department which greatly facilitated this study.

SUMMARY. Investigations in Columbus, Georgia and other urban areas of the state during the fall and winter of 1955–1956 demonstrate that both breeding and biting activities of *Culex quinquefasciatus* continue during every month from October through March. The relationship of this species to human welfare is briefly discussed.

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NOTES ON *AEDES TRISERIATUS* EGG INCUBATION AND COLONIZATION

RICHARD O. HAYES and HARVEY B. MORLAN[1]

Aedes triseriatus (Say) has been found to be a natural or laboratory vector of bird malaria (Huff, 1932), dog heartworm (Phillips, 1939), and eastern equine encephalitis (Davis, 1940). The development by Repass (1952) of a laboratory technique for colonizing this species has greatly enhanced its usefulness in laboratory research. However, several instances in which low percentage egg hatches jeopardized the existence of established laboratory colonies have been brought to the attention of the authors. Baker (1935) reported a case in which the hatching of *A. triseriatus* was apparently influenced by the amount of daylight available and he believed the photosensitive ova would not hatch unless exposed to sufficient light. Love and Whelchel (1955) obtained no indication of this effect and concluded that the reduction of the photoperiod in the fall influenced only the time required for the completion of larval development. The present study of the requirements for egg development, hatching, and conditioning was undertaken to determine the means of insuring consistently satisfactory *A. triseriatus* egg hatches.

Methods. The *A. triseriatus* colony was established with larvae collected from artificial containers in Bonaventure Cemetery, Savannah, Georgia, and was maintained in a cage (22 x 22 x 22 inches) at 75 ± 5° F. and 75 ± 10 percent relative humidity. The cage was screened on three sides and the top with 20-mesh plastic screen. The back and the floor were 0.5 inch plyboard. A screened tunnel (6.5 x 7 x 22 inches) extended from the front to the back of the cage about 8 inches above the floor on the left side. A platform hinged to the back of the cage, and 2 inches above the floor of the tunnel, extended through the tunnel to the front of the cage. An opening, 7 x 9 inches, was provided in the lower right front corner of the cage for the attachment of a cloth sleeve.

A blood meal was offered to the adults for 3 hours each day by placing a rabbit in a 21 x 6 x 1.5 inch metal tray on the tunnel platform which was then wedged upward to force the clipped back of the rabbit against the 20-mesh screen of the tunnel. The adult female mosquitoes readily fed through the screen. A wood block, 6 x 5.5 x 1.5 inches, placed in the tray beneath the rabbit's abdomen forced the rabbit's back against the screen. The outer end of the tray, 3.5 inches high, served as a barrier preventing the rabbit's escape. This type of cage eliminated the loss of mosquitoes usually encountered while placing an animal into, and removing it from, a stock cage. Seedless raisins, a pad soaked with a one-molar sucrose solution, and water were provided fresh each week as mosquito nourishment.

From September through mid-January, an automatically-operated light system furnished a 45-minute evening crepuscular period followed by 10 hours of darkness.

The use of saturated paper toweling as an oviposition site, instead of chips or blocks of wood as devised by Repass (1952), facilitated egg handling and storage. Eggs were laid upon a strip of moist paper toweling 3 inches wide, lining an uncovered 600-ml. beaker containing about 200 ml. of water. The water and the toweling were placed in the beaker approximately 24 hours before being placed in the colony cage.

To determine the time needed for completion of embryonic development, eggs

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of known ages were tested at 79 ± 3° F. Beginning on the third day with a given egg batch and continuing through the eighth day, replicate lots of about 100 eggs were removed on successive days from the oviposition beakers and immersed in tap water. After submergence in tap water for 24 hours, the percentage of egg hatch was determined. All unhatched eggs were dissected, and the degree of embryonic development recorded.

Tests were conducted to compare the percentage of hatch obtained among eggs immersed in tap water and in other media. In preliminary trials, the following media were tested: 50 milligrams of ground dog chow per liter of water, and 100 milligrams, each, of ground dog chow and of yeast per liter of water. Subsequent tests compared the percentage of hatch obtained in four mixtures of dog chow and yeast in water. The proportions of dog chow to yeast were 50:50, 100:50, 50:100, and 100:100 milligrams per liter of water. The eggs from a single batch were divided into four approximately equal groups and were submerged in one of the four media for either 18 or 22 hours. In any given test the eggs in each of the four groups were submerged for the same time period. All unhatched eggs were dissected to determine the percent viable.

Dissection of the eggs was accomplished under a dissecting microscope using *minuten Nadeln* attached to applicator sticks. To prevent the eggs from rolling during the dissection, they were placed in drops of clean water on the adhesive surface of drafting tape attached, adhesive surface up, along the length of a 1 x 3 inch microscope slide.

Each dissected egg containing a larva showing complete embryonic development and movement was considered viable. Well developed embryos in which the cephalic egg breaker was darkly pigmented constituted the criterion for complete embryonic development.

Eggs from given batches were matured under different relative humidities at 79 ± 3° F. to determine which would give the more satisfactory hatches. Hatches obtained in paired tests utilizing approximately equal numbers of eggs from a given egg batch were first compared with eggs incubated at relative humidities of 70 and 80 percent and later with eggs incubated at relative humidities of 80 and 90 percent.

The 70, 80, and 90 percent relative humidities, maintained with saturated salt solutions of sodium nitrate, potassium bromide, and barium chloride, respectively, varied ± 2 percent. Eggs were subjected to a saturated atmosphere by placement on moist paper toweling in covered oviposition beakers. The effect of the direct contact of the toweling with water may be important, but its significance was not tested.

On each successive day following oviposition, the saturated toweling containing a batch of eggs laid in a single day was removed from the oviposition beaker. Two strips of toweling, each containing about 100 eggs, were cut from the egg batch. Each of the strips was hung on a rack in a chamber maintaining one of the desired relative humidities. The remainder of the egg batch was returned to the saturated atmosphere in the oviposition beaker. Thus a series was obtained in which 6-day-old and 8-day-old eggs had been incubated at saturation, at either 70 or 80 percent relative humidity, and at all of the possible daily combinations of 70 percent relative humidity and saturation, or 80 percent relative humidity and saturation. Following storage at each of the test conditions, the percentage hatch of 6-day-old and of 8-day-old eggs was determined by immersing them for 22 hours in equal quantities of a standard hatching medium. The percentage of hatch was based upon egg and larval counts made at the end of the immersion period.

At weekly intervals, samples of eggs were taken from a given batch to determine the effect of aging on the percentage of hatch. Ten egg batches, each laid

in successive weeks, were used. Two batches contained only enough eggs for testing four consecutive weeks, and no samples from any batch were tested beyond the eleventh week. Although attempts were made to use 100 eggs in each weekly sample, the variation in egg density and the number of eggs per batch resulted in samples ranging from 32 to 340 eggs with a mean of 106 eggs for the 63 samples tested. All of the eggs were incubated for 6 days in the oviposition beaker before being stored at 80 percent relative humidity. The temperature for incubation, storage, and testing was 75 ± 5° F. In each test, the eggs were submerged for 4 hours in equal quantities of a standard hatching medium.

Results. Mating in the colony was observed to occur during periods of semi-darkness. The males grasped the females during flight, the pair fell to the floor of the cage, and mating was completed in about 5 seconds.

Oviposition occurred principally at night. Egg batches ranged in size from 159 to 1,120 eggs.

At 79 ± 3° F. a minimum of 4 days was found necessary for complete development of the *A. triseriatus* embryo. Six days was the minimum time in which complete development by all the eggs within a batch resulted in a 100-percent hatch. Based on the number of viable eggs, averages of 0, 43, 48, 74, 75, and 95 percent hatched when 3 through 8 days old, respectively; based on the total number of eggs, averages of 0, 19, 18, 56, 16, and 78 percent hatched.

Preliminary trials showed that 1-day-old mixtures of dog chow and brewers' yeast in water stimulated hatching more than fresh or 1-day-old media of tap water, dog chow in water, yeast in water, or fresh mixtures of the dog chow and yeast in water. On the basis of 12 tests of four different dog chow-yeast combinations, the mixture containing 0.2 gram each of ground dog chow and of powdered brewers' yeast per liter of water yielded the largest mean percentage hatch of viable eggs and of total eggs. A 1-day-old formulation of this mixture was adopted as the standard hatching medium.

No hatches greater than 30 percent were obtained in 38 replicate tests obtained from either 6- or 8-day-old eggs incubated continuously at 70, 80, or 90 percent relative humidity. These poor hatches emphasized the need for an incubation period in a saturated atmosphere. Six-day-old eggs incubated continuously in a saturated atmosphere averaged 78 percent hatch, the best average recorded for that age group; the 8-day-old eggs continuously held in a saturated atmosphere averaged 75 percent hatch, but this was not the highest average hatch among this age group.

Six- and 8-day-old eggs incubated for a portion of their prehatching period at 80 percent relative humidity consistently yielded larger mean percent hatches than eggs incubated for similar periods of time at either 70 or 90 percent relative humidity. Nineteen replicate paired tests compared hatches between 6-day-old eggs incubated for known portions of their prehatch period at either 70 or 80 percent relative humidity and at either 80 or 90 percent relative humidity. An average 69 percent hatch of eggs, conditioned 5 days in the saturated atmosphere of the oviposition beaker and 1 day in the 80 percent relative humidity chamber, was the best result obtained in the series. In a similar series with 8-day-old eggs, the best mean hatch of 90 percent was obtained from eggs incubated 6 days in the saturated atmosphere of the oviposition beaker and 2 days in the 80 percent relative humidity chamber. Eight-day-old eggs yielded 100 percent hatches in two cases. One of these was with eggs incubated 7 days in the oviposition beaker and 1 day in the 80 percent relative humidity chamber and the other was incubated under the preceding conditions for 5 and 3 days, respectively.

Egg hatch decreased as egg age increased beyond 2 weeks. An average of 75 percent of the eggs 1 and 2 weeks old

? 0.1 gm. each (ie 0.2 gm

hatched. Slightly over 50 percent of all the eggs 3 through 8 weeks old hatched but only 33 percent of all the eggs 9 through 11 weeks old hatched. However, in one egg batch, hatches averaging 92 percent per trial occurred through the eighth week. The first trial in which no hatch was obtained occurred during one of the two trials with 11-week-old eggs.

The larvae were fed Purina dog chow which had been ground and sifted through a 40-mesh screen. Ten to 14 days usually were required for development from the egg to the adult stage. Crowding, overfeeding, underfeeding, or reduction of temperature increased the time required for larval development.

SUMMARY. A self-sustaining laboratory colony of *A. triseriatus* was readily established and maintained from field-collected larvae. Oviposition upon moist paper toweling showed the use of wooden blocks for oviposition sites to be unnecessary. A technique utilizing the adhesive surface of drafting tape to prevent eggs from rolling during dissection is described.

A minimum of 4 days at approximately 80° F. was found necessary for the development of the embryo, and 6 days was the minimum time in which complete development by all the eggs in a batch provided a 100 percent hatch.

The percentage of egg hatch was increased by hatching eggs in day-old media consisting of 0.2 gram each of ground dog chow and of powdered brewers' yeast per liter of water.

The need for a period of incubation in a saturated atmosphere (over water on a moist substrate) was indicated by the low hatches obtained in paired tests of 6- and 8-day-old eggs incubated continuously at about 80° F. and 70, 80, or 90 percent relative humidity.

Six- and 8-day-old eggs matured for a portion of their prehatch period at 80 percent relative humidity consistently produced larger hatches than those which had been incubated a similar portion of their prehatch period at either 70 or 90 percent.

A 78 percent hatch of 6-day-old eggs incubated continuously in a saturated atmosphere was the best average recorded for that age group. The best mean hatch of 8-day-old eggs, 90 percent, was obtained from eggs incubated the first 6 days in a saturated atmosphere and the last 2 days in 80 percent humidity.

A reduction in the percentage of eggs hatching was found to occur when egg age exceeded 2 weeks.

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41 106

IDENTIFICATION OF BLOOD MEALS OF BLACKFLIES (DIPTERA: SIMULIIDAE) ATTACKING FARM ANIMALS [1]

A. E. R. DOWNE [2] AND P. E. MORRISON [3]

The development of serological methods to determine the sources of blood meals of haematophagous insects has stimulated studies of host preference and multiple-feeding habits of biting flies in many parts of the world. Such studies have been conducted chiefly in connection with problems of disease transmission. There are, however, few records of attempts to study the host ranges or host preferences of biting flies in Canada. Rempel *et al.* (1946) reported precipitin-test studies of the feeding habits of *Aedes* mosquitoes in Saskatchewan, and Downe and West (1954) gave a brief account of the blood meals of *Aedes hexodontus* Dyar at Churchill, Manitoba. We have found no published accounts of serological studies of the blood meals of Canadian species of Simuliidae.

This report describes preliminary studies on determining the blood meals of blackfly species attacking domestic animals on a farm near Rapides des Joachims, Quebec, during the spring and summer of 1954.

Collection of Blackflies. Blood-engorged blackflies were collected in window traps in a one-room barn housing horses, cattle, pigs, and chickens. The traps were patterned after the designs of various authors and have been illustrated by Peterson (1955). While the blackflies were abundant, specimens were removed from the traps early in the morning and at dusk. Later collections were made once daily. Tests had shown that blood meals of blackflies can be determined by the precipitin test when they have remained in the guts of the living insects not more than 24 hours. The specimens used in the test included only blood-engorged individuals that were collected during 24-hour periods when exposure to blackflies was about equal for the animals. Specimens removed from the traps were killed with ether or chloroform, and about half of them were mounted on insect pins, the remainder being smeared on pieces of filter paper. All pinned specimens were determined to species with the assistance of Mr. G. E. Shewell, Entomology Division, Ottawa. Smeared specimens were classified only as blackflies.

Serological Methods. Preparation of Antiserum. Antisera were prepared in healthy adult rabbits by injecting the sera of various domestic animals and of humans by the method of Proom (1943). Seven to 10 days after inoculation, each rabbit was trial-bled from the ear, and the serum tested for antibody sensitivity with the homologous antigen as described by Eligh (1952). The titre was determined as the highest dilution of serum antigen that gave a positive precipitin-ring test with the constant dilution of antiserum. If titres from trial bleedings were sufficiently high (1:5,000 or higher), larger quantities of blood were collected from the rabbits by ear bleeding or cardiac puncture. After the blood had clotted, antiserum was removed, sterilized by Seitz filtration, and stored in sterile bottles at —25° C. until required for testing the biting flies. Many of the antisera were observed to be nonspecific: anti-horse serum often reacted with dilutions of pig serum and beef serum, and anti-pig and anti-horse sera gave cross reactions also (cf. Weitz, 1952). The extent of cross reactions was determined for a sample of each antiserum, and cross reactions were eliminated by absorption techniques described by Weitz (1952). The anti-human serum did not react with the antigens of species other than man. Anti-chicken

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serum produced in rabbits is nonspecific and detects all avian antigens (Downe and West, 1954; Eligh, 1952). No attempt was made to render the anti-chicken serum specific, but no cross reactions with mammalian sera were observed.

PRECIPITIN TESTING OF BITING FLIES. Extracts were prepared from the dried blackflies mounted on pins as well as from the filter-paper smears. The insects were removed from the pins and crushed with sterile forceps in 5-ml. beakers. One millilitre of physiological saline was added to each beaker and extracted for three to four hours at room temperature. The extracts were transferred in 0.05-ml. amounts to small tubes (3 mm. in diameter by 25 mm. long) so that each extract was tested for horse, beef, pig, chicken, and human blood. Antisera were dyed with safranine "O" as described by Herndon and Schubert (1951) before being layered beneath extracts. A positive reaction was indicated by the appearance of a precipitin ring at the interface between the extract and the antiserum. The smears were extracted and tested in a similar manner.

The precipitin testing of dried specimens (Amaral and Aguiar, 1950) has some advantages over the use of filter-paper smears. The smears must be prepared shortly after the insects have been trapped in the field whereas pinned specimens may be retained for identification. This is of particular value with species difficult to identify in the field. Our tests showed that the results obtained with dried specimens are as accurate as those with smears.

RESULTS AND DISCUSSION. The data in

TABLE 1.—Numbers of blackfly species that gave positive precipitin reactions for various domestic animals.* The blackflies were collected during 24-hour periods from a one-room barn housing the animals.

	Animals in barn				
Species	2 Horses 1 Cow 2 Periods	2 Horses 2 Cows 4 Chickens 6 Periods	2 Horses 4 Cows 2 Pigs 14 Chickens 17 Periods	2 Horses 3 Cows 2 Pigs 14 Chickens 3 Periods	Totals
Simulium vittatum Zett.	29 H	58 H	201 H	19 H	307 H
		3 B	59 B	1 B	63 B
			2 P		2 P
S. venustum Say	17 H	59 H	164 H	26 H	266 H
		11 B	42 B	6 B	59 B
		1 X			1 X
S. parnassum Mall.		9 H	43 H	7 H	59 H
		2 B	16 B	1 B	19 B
S. decorum Wlk.			24 H	3 H	27 H
		1 B	26 B	5 B	32 B
S. tuberosum Lund.			5 H	1 H	6 H
		1 B	3 B		4 B
			1 F		1 F
S. corbis Twinn			5 H		5 H
			2 B		2 B
	1 X		1 X		2 X
S. rugglesi N. & M.	1 F				1 F
Prosimulium hirtipes (Fries)	2 H		27 H	4 H	33 H
	2 B		16 B		18 B
			1 P		1 P
Cnephia mutata (Mall.)			8 H		8 H
			6 B		6 B

* B, beef; F, bird; H, horse; P, pig; X, undetermined feeding.

Table 1 (the results for the pinned specimens) show that pigs and chickens were seldom attacked when horses and cattle were also present. *Simulium vittatum* and *S. venustum* attacked horses much more frequently than cows, and most of the other species attacked horses more frequently than the other animals. No positive reactions were obtained with anti-human serum; humans were in the barn only for short periods while handling the livestock or collecting specimens. The small number of undetermined feedings (0.3 percent) suggests that there was little possibility of other hosts, such as small game or wild birds, being involved.

None of the specimens tested contained blood from more than one host, indicating that multiple feeding may be uncommon among blackflies. Tests of blackfly smears appear to support this statement. Of 750 smears tested, 518 were positive for horse, 221 for beef, and 5 for pig; 5 gave no reaction and only one was positive for both beef and pig. Blackflies are not readily disturbed once they start feeding and tend to remain on one host until fully engorged.

The results suggest that certain blackflies have decided host preferences and that pigs and chickens are rarely attacked by any of the species listed in Table 1. For some of the species, the numbers collected were too small to attach much significance to the findings. Records of blackflies attacking pigs are found in the literature, and, in western Canada, Rempel and Arnason (1947) reported that *Simulium arcticum* Mall. will attack pigs but prefers cattle and horses.

Several of the species listed in Table 1 have been recorded as feeding on birds. *Simulium rugglesi* is a well-known bird feeder and the role of this blackfly in disseminating *Leucocytazoon simondi* M. & L. in ducks and geese in Canada has been discussed by Shewell (1955). Fallis *et al.* (1951) reported that *Simulium venustum, S. vittatum, S. decorum, S. tuberosum, S. parnassum,* and *Prosimulium hirtipes* can be induced to feed on ducks in the laboratory. The present studies show that all these species except *S. rugglesi* obtain blood meals from mammals even when birds are present.

Some approaches to studies on host preferences of blackflies are made difficult by certain habits of the insects. They appear to be very sensitive to unnatural conditions, and attempts to induce them to feed on domestic animals in cages or in bait traps were largely unsuccessful. Blackflies (particularly *Simulium vittatum, S. venustum,* and *S. decorum*) readily entered animal-baited traps through baffle entries, but once inside they ignored the host and searched for a means of escape.

Summary. The blood meals of blackflies attacking domestic animals on a farm near Rapides des Joachims, Quebec, during the summer of 1954 were determined by the precipitin test. Horses and cattle were attacked by all but one of the species studied, but pigs and chickens were seldom bitten. Of 1,672 specimens tested, only one (a smeared, unidentified specimen) had fed on more than one host. Some limitations of host-preference studies of blackflies are discussed.

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ADDITIONAL STUDIES ON THE DISTRIBUTION OF MOSQUITO LARVAE AND PUPAE WITHIN A RICE FIELD CHECK

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The data presented in this paper are a continuation of studies conducted in the San Joaquin Valley (Stanislaus County), California in 1947 (Markos, 1951). In order to corroborate the 1947 findings, similar studies were conducted in 1949 in the rice field areas of the Sacramento Valley, which is the foremost rice growing area in the state.

For this study a large rectangular rice check was selected at the Biggs Rice Experiment Station, Butte County. The field was of a Stockton clay adobe soil, well suited for a study of this type. This study was one of several undertaken in the various rice checks located at the Biggs Rice Station.

METHODS. The procedures followed in this investigation were essentially the same as those used in the previous study and have been described in detail (Markos, 1951).

When completely flooded, the Biggs rice check comprised an area of 38,564 square feet, its dimensions being 124 x 311 feet.

In order to compare the relative density of mosquito larvae across the middle of the check with that occurring along the levees an equal number of dips, 104 across the middle and 104 along the levees, were taken. The mid-field of the study check was defined as the area directly across the bridge, excluding the first twelve feet which was the length of the first plank at either end. The numbered levee stations were in turn subdivided into so-called A, B, and C sections of the stations, as in the previous study.

The study check was inspected three times each week (Monday-Wednesday-Friday) from the time of flooding to the end of the season.

OBSERVATIONS. The algal growth in the study check consisted of *Oedogonium* spp. and *Nostoc* along with *Calothrix, Scenedesmus, Anabaena* and *Zygnema.*

Among the more common weeds which appeared in the check were the following: barnyard grasses (*Echinochloa crusgalli* and varieties), cattails (*Typha latifolia*), water plantain (*Alisma plantago-aquatica*), arrowhead (*Sagittaria latifolia*), spike rush (*Eleocharis palustris*), redstem (*Ammannia coccinea*), slim aster (*Aster exilis*), water hyssop (*Bacopa rotundifolia*), bulrush (*Scirpus fluviatilis*), umbrella plants (*Cyperus*), and *Najas*, a very prevalent waterweed found below the water surface. The check contained an excellent

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growth of rice, and the weeds which were present were scattered in small numbers throughout the check.

The rice was in seed during the fourth week of April. Observations on May 18, after the rice plant had emerged from the water, showed a mean density of 8.35 plants per square foot along the center, and 7.89 plants per square foot along the levees.

Water flowed in and out of the check during most of the season. The fluctuation is shown in Figure 1.

The mean height of the vegetation (principally rice plants) is indicated in Table 1.

A total of 12,197 dips were taken during the season from April to September from the entire check. Of these, 6,136 dips were from the center and 6,061 were from the levees. This discrepancy was due to the lowering of the water in the study check, which caused a few of the levee stations to become dry.

After the check had undergone the usual preliminary land preparation including plowing and disking it was irrigated on April 25 and completely flooded April 26. Observations on April 26 and 29 revealed no mosquito larvae. On May 2, however, a few *Aedes* larvae were noted for the first time. Subsequent observations showed a steady increase, reaching a peak in density on May 6. By May 11 *Aedes* larvae and pupae could no longer be found in the check.

Aedes larvae appeared to be much more prevalent along the levees or margins. This was at least in part due to the

FIGURE 1
FLUCTUATION OF WATER IN BIGGS RICE CHECK
APRIL - SEPTEMBER, 1949

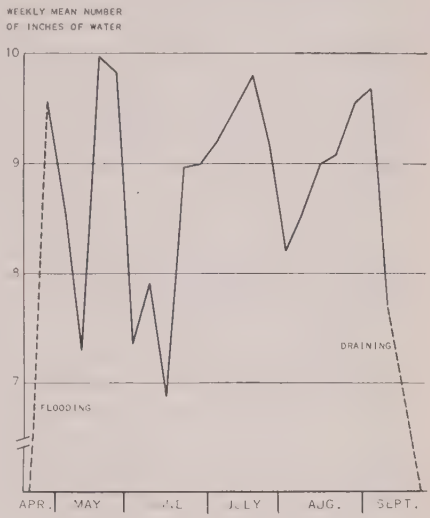


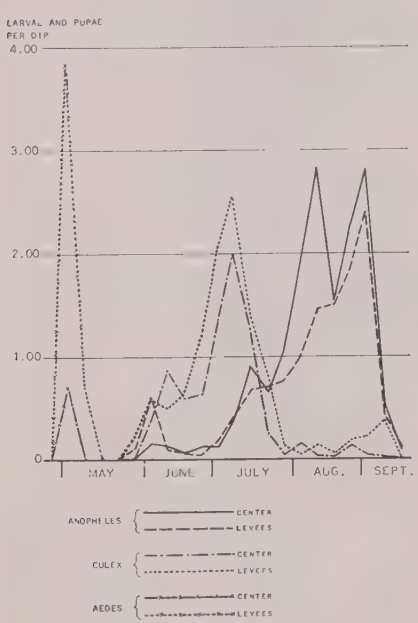
TABLE 1.—Mean height of vegetation (in inches) along center and levees of Biggs rice check April–September, 1949

Week of Observation	Total Number of Stations	Mean Height of Vegetation (in inches) Center [1]	Total Number of Stations [2]	Mean Height of Vegetation (in inches) Levees [1]
April				
24–30	104	Seed	104	Seed
May				
15–21	104	13.82	104	11.44
June				
12–18	104	17.49	104	17.41
July				
17–23	104	26.24	104	28.60
August				
14–20	104	34.26	104	38.17
September				
11–17	104	38.82	104	42.93

[1] Vegetation principally rice.
[2] Actually the mean of all dipping stations (104A, B and C stations) along the levees.

strong winds which prevailed in the area at this time. Adults reared from numerous larval and pupal collections consisted of *Aedes melanimon* Dyar.[3] This heavy breeding of the *Aedes* phase lasted for only two weeks. No *Aedes* specimens were found in the check during the remainder of the season. The seasonal prevalence of the *Aedes* phase is indicated in Figure 2. There was no vegetation in the check at this time.

FIGURE 2
ANOPHELES, CULEX AND AEDES FROM CENTER AND LEVEES OF RICE EXPERIMENT STATION CHECK
APRIL - SEPTEMBER, 1949



The weekly mean number of *Culex* larvae and pupae from the center and levees is indicated in Figure 2. It is apparent that *Culex* mosquitoes were well distributed within the check. *Culex* species reached their peak during the second week of July and then quickly diminished to very small numbers during the remainder of the season. The dominant *Culex*, as determined by reared adults and larval identifications, was *Culex tarsalis* (Coquillett).

In the late summer *Anopheles* species dominated the rice check. The weekly mean number of *Anopheles* larvae and pupae from the center and levees is shown in Figure 2. From their first appearance early in the season *Anopheles* slowly increased in numbers to attain a maximum density during August and early September. The dominant representative of this genus was *Anopheles freeborni* Aitken.

Only two additional species of mosquitoes were found in the rice check during the season. On June 24 and July 6 a few *Culex erythrothorax* Dyar were taken from bridge stations in the check. A few adults of *Anopheles franciscanus* McCracken were reared from collections taken from levee stations on August 1.

DISCUSSION. It is of significance to compare the seasonal incidence of the mosquito larvae in the rice check to the south in the San Joaquin Valley (Stanislaus County), with that in the north in the Sacramento Valley (Butte County). These two similar studies were carried out in 1947 and 1949, respectively, in areas widely separated in California. Rice is grown in California principally in the Sacramento Valley and to a much lesser extent in the San Joaquin Valley.

The *Aedes* phase occurred between April 14–28, 1947, in the rice fields in the San Joaquin Valley, and between April 26–May 9, 1949, in the check in the Sacramento Valley. It is logical to expect that the *Aedes* phase would occur at approximately the same time even in rather widely separated areas, inasmuch as its appearance is closely related to the time of flooding of the rice fields.

There appeared to be only a slight variation in the seasonal prevalence of the *Culex* phase. In the San Joaquin Valley the *C. tarsalis* reached its peak between the last week of June and first week of July; in the Sacramento Valley

[3] Identified at the time as *Aedes dorsalis* (Meigen) in keeping with the then current taxonomic understandings.

check it reached its peak during the second week of July.

There also appears to be only a slight variation with *Anopheles* in the two areas. In San Joaquin Valley rice fields *A. freeborni* reached its peak during the first week of August and second week of September; in the Sacramento Valley it reached its peak between the third week of August and first week of September.

The seasonal incidence of *A. freeborni* in the interior valleys of California has been discussed by Freeborn (1932) who, basing his study on a correlation of adult and larval population, found that in the Sacramento Valley there are six generations a year. Larval collections were made at the same time from aquatic habitats adjacent to the collection points for adults. These habitats consisted of pools of water adjacent to rice field areas. Freeborn showed the seasonal abundance of adults and larvae of *A. freeborni* to increase steadily from May to October, with apparent peaks during the third week of May, fourth week of June, fifth week of July, third week of September, and the first week of October.

In the San Joaquin Valley rice fields we found a steady increase of *A. freeborni* in the checks from May to September, with apparent peaks during the first and fourth week of June, first week of July, first and third week of August, and the second week of September. In the Sacramento Valley it was also observed that there was a steady increase from June to September, with well-defined peaks occurring during the second week of June, the third week of July, the third week of August and finally during the first week of September.

Summary. Observations in a rice field check at the Biggs Rice Station in Butte County, California during the summer of 1949, revealed that:

1. The rice check was productive of considerable numbers of mosquitoes which appeared in three "phases." The *Aedes*, represented by *A. melanimon*, appeared immediately after flooding and then disappeared to be followed by *C. tarsalis* and *A. freeborni*.

2. The *A. melanimon* population reached its maximum during the first week of May, *C. tarsalis* during the second week of July, and *A. freeborni* during late August and the first week of September.

3. In addition to the above species, small numbers of *C. erythrothorax* were taken from the check center during the fourth week of June and first week of July. *A. franciscanus* was taken during the first week of August along the levee.

4. The center of the rice check was found to be as productive of mosquito larvae as were the areas adjacent to the levees. Larvae of the above-mentioned species were found to be well distributed throughout the check, except for the *Aedes* which were found in greater abundance along the levees.

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TABANIDAE OF THE TENNESSEE VALLEY WATERSHED[1]

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A survey of insects of public health significance occurring in the vicinity of TVA reservoirs was undertaken in fiscal years 1953 and 1954. Included in this survey were mosquitoes, punkies, deer flies, horse flies, blackflies, and stable flies. It is the purpose of this paper to present information on the distribution and biology of the deer flies and horse flies encountered in this survey as well as records from incidental collections prior to 1953. The first and only extensive collections in the Valley before the 1953–1954 survey were made in north Alabama by Dr. John N. Belkin in 1941 and 1942 while on the TVA staff. We are indebted to him for the use of these records in this paper.

Although the Tennessee River watershed extends into seven Southeastern States, its main reservoirs are largely confined to Alabama and Tennessee. The majority of the collections was made in forested and pastured portions of the floodplain in these states. In several instances populations of tabanids were sampled along tributary streams in mountainous areas of the watershed included in the Cherokee National Forest. At Greenbrier, Alabama, deer flies were observed in a tupelo gum swamp maintained at more or less constant pool level by beaver dams. Heavy concentrations of deer flies were also noted in sandy creek bottoms near Bruceton and Paris, Tennessee, where highway and railroad construction has caused marshy and swampy situations to develop. Records of species caught in connection with observations on biting Diptera in a cypress forest at Reelfoot Lake, Tennessee, are also included.

Since the tabanids do not generally constitute a source of annoyance to man and animals in reservoirs of the Tennessee River Valley, no extended efforts were made to determine larval breeding situations. Larvae and pupae of *Leucotabanus annulatus* Say were collected on several occasions from moist tree cavities in connection with studies on larval breeding sites of punkies. In one instance, a tabanid larva, presumed to be *Tabanus atratus* Fab., was taken while actively moving just beneath the surface of a large sulphate waste basin near Calhoun, Tennessee.

Adult populations were sampled while on animal hosts, in natural resting sites, parked and moving cars, light traps, rotary trap, on windows, and by net sweeping. Black panel trucks with fairly deep (2½″–3″) side window casings were especially suited to the detention of tabanids entering through the rear door when the vehicles were parked along pastured reservoir margins. The following combinations of letters are employed in the distribution records to indicate the method or conditions pertaining to the collection: BC (biting a cow), BH (biting a horse), BM (biting a man), BP (biting a pig), BU (biting a mule), C (interior of car or truck) LT (light trap), RT (rotary trap), and S (net sweeping).

The distribution records presented in this paper include 51 species and 7 subspecies in the following genera: *Chrysops* (22), *Hamatabanus* (1), *Leucotabanus* (1), and *Tabanus* (34). The genus *Chlorotabanus* is very probably present in the Valley since Pechuman (1954) records a collection of *C. crepuscularis* (Bequ.) by

[1] Acknowledgment is made to Dr. Gordon E. Smith, Chief Biologist, Division of Health and Safety, for his guidance and helpful suggestions during the course of this survey. Special thanks are due Dr. Alan Stone, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, for identification of specimens and checking the manuscript list of species.

Benesh at Burrville, Tennessee, in the nearby Cumberland watershed. Of the 1,160 specimens collected, the majority (773) were deer flies (*Chrysops* spp.). This plurality of *Chrysops* suggests that deer flies are more anthropophilic and hence are more frequently encountered, that they are more restricted in the environment and show greater numbers in localized situations, or perhaps indicate a greater reproductive potential. The 387 horse flies were distributed as follows: *Hamatabanus* (2), *Leucotabanus* (15), and *Tabanus* (370).

Chrysops beameri Bren. Tennessee: Hollow Rock, VIII–21–53 (C–1).

Chrysops brimleyi Hine Tennessee: Newport, V–11–54 (BH–1).

Chrysops brunnea Hine Tennessee: Samburg, VI–15–51 (C–1) (BM–4), VI–16–51 (C–3), VI–14–52 (BM–2), VI–16–52 (BM–2), VI–17–52 (BM–2, VI–18–52 (BM–2), VIII–22–52 (BM–2), VIII–23–52 (BM–1), VII–22–54 (BM–54) (C–51), VII–23–54 (LT–1). Adults of *C. brunnea* have been frequently observed biting man during the daylight hours in and along the margin of a cypress swamp on Bayou du Chien near Samburg at Reelfoot Lake, Tennessee. Feeding on man in the forest has been most pronounced in the late afternoon just before dusk, particularly in the shrub layer of vegetation.

Chrysops callida O. S. Tennessee: Camden, VI–9–53 (BM–1), VI–23–53 (C–1), VII–6–53 (BM–1), V–18–54 (BM–2), VII–7–54 (BM–2), VII–29–54 (C–1). Alabama: Decatur, V–28–41 (4), VI–9–41 (21), VI–21–41 (4); Mooresville VI–3–41 (1); Town Creek, V–29–41 (2); Triana, VI–9–41 (9), V–28–42 (1); Greenbrier, VI–19–41 (1); Florence, VII–1–41 (12); Wilson Dam, V–27–42 (1), VI–15–42 (3), VI–26–42 (1), VII–1–42 (4); Whiteside, V–28–42 (1); Rogersville, VII–2–54 (BM–1). North Carolina: Bryson City, VII–1–53 (C–1).

Chrysops carbonaria Walk. Tennessee: Sugar Tree, V–14–52 (C–1); Parksville, V–24–54 (C–1).

Chrysops cursim Whit. Tennessee: Paris, VI–22–54 (C–1).

Chrysops dacne Philip Tennessee: Camden, VII–6–53 (BM–3), VII–7–53 (BM-2), V–10–54 (C–1), VII–1–54 (C–1), VII–7–54 (C–1); Paris, VI–3–54 (C–2), VI–23–54 (S–23) (BM–21); Hollow Rock, VI–22–54 (BM–4), VII–8–54 (S–2); Springville, VI–22–55 (C–1); Lake City, VII–14–55 (BM–1). Alabama: Decatur, VI–9–41 (16), VI–11–41 (16), VI–21–41 (3), VI–26–41 (1); Greenbrier, VI–20–55 (C–2).

Chrysops flavida Wied. Tennessee: Oak Ridge, VII–23–49 (S–1); Delano, IV–18–52 (S–1); Paris, VI–3–53 (BM–1); Pickwick Dam Village, VI–23–53 (BM–1); Camden, VI–24–53 (C–1), VII–6–53 (BM–1), VII–7–53 (BM–3), VIII–3–53 (BM–3), VIII–14–53 (C–2), VIII–15–53 (C–3) (BM–3), VIII–16–53 (BM–2), VIII–22–53 (C–4), VI–22–54 (C–1), VII–1–54 (C–1), VII–29–54 (C–1), VIII–26–54 (C–12), IX–10–54 (C–1); Hustburg, VII–20–53 (LT–1), VIII–17–53 (LT–1), VI–18–54 (LT–1); Samburg, VIII–16–53 (BM–2), VII–22–54 (BM–2); Eva, VI–23–54 (C–1); Faxon, VI–24–54 (C–1); McKinnon, VI–24–54 (C–1), VI–28–54 (C–1). Alabama: Decatur, VI–3–41 (1), VI–11–41 (65), VI–21–41 (1); Wilson Dam, VII–1–42 (1), VI–16–54 (BM–1); Rogersville, VII–2–54 (BM–3), VII–9–54 (BM–3), VII–16–54 (BM–3); Florence, VII–13–54 (C–1); Sheffield, VII–14–54 (C–1); Madison, VIII–25–54 (C–5); Greenbrier, V–6–55 (BM–1), VI–20–55 (C–4), VII–15–55 (C–4), VII–18–55 (RT–1), VII–24–55 (C–1), VII–26–55 (C–3), VII–27–55 (C–2), VIII–9–55 (C–1), VIII–10–55 (C–1), VIII–18–55 (RT–1), VIII–19–55 (C–1.) This species was the most pestiferous deer fly observed in north Alabama. It was particularly abundant in tupelo gum swamps from June to August. In Tennessee, it was commonly encountered with *Chrysops wiedemanni* along reservoir margins colonized by buttonball (*Cephalanthus occidentalis*).

Chrysops flavida celata Pech. ALABAMA: Decatur, VI–11–41 (3).

Chrysops flavida reicherti Fair. TENNESSEE: Samburg, VIII–23–41 (1), VI–15–51 (BM–4), VI–16–51 (C–3), VI–17–51 (BM–8), VI–18–51 (BM–1), VI–15–52 (BM–1), VI–17–52 (BM–22), VI–18–52 (BM–1), VIII–20–52 (BM–4), VIII–21–52 (BM–2), VIII–25–52 (BM–1). ALABAMA: Triana, VI–9–41 (2); Decatur, VI–11–41 (6), VI–21–41 (1); Wilson Dam, VI–15–42 (9). Along with *Chrysops brunnea,* this species was annoying to man along the margins of Bayou du Chien at Reelfoot Lake, Tennessee. In addition to bald cypress the bayou is margined with dense stands of giant sawgrass (*Zizaniopsis miliacea*).

Chrysops geminata Wied. TENNESSEE: Hollow Rock, VI–22–54 (BM–1); Parksville, VI–23–54 (BM–1). NORTH CAROLINA: Andrews, VII–7–54 (C–1).

Chrysops moecha O. S. TENNESSEE: Paris, VII–29–53 (C–1), VI–7–54 (C–3); Camden, V–10–54 (C–1), VI–11–54 (C–1); Gallatin, VI–11–54 (C–1). ALABAMA: Mooresville, VI–9–41 (1).

Chrysops montana O. S. ALABAMA: Decatur, V–28–41 (1), V–28–42 (4).

Chrysops nigra Macq. TENNESSEE: Sugar Tree, V–14–52 (BM–2), V–23–53 (BM–1), V–25–53 (BM–1); Camden, V–21–53 (BM–11); VI–8–53 (BM–2) (C–1), VI–9–53 (BM–3), V–28–54 (C–2), VI–18–54 (C–1), VII–7–54 (C–1); Englewood, IV–30–54 (BM–2); Tellico Plains, IV–30–54 (BU–1); Big Sandy, V–11–54 (C–1); Hollow Rock, V–17–54 (C–1); Carderview, VI–9–54 (BH–1). ALABAMA: Decatur, V–28–41 (1); Florence, V–23–54 (C–1); Fort Payne, VI–22–54 (BM–1); Town Creek, IV–29–55 (BM–2). Adults of *C. nigra* were especially numerous in a black willow-cattail bottom near Camden, Tenn.

Chrysops parvula Daecke. TENNESSEE: Paris, VI–23–54 (S–1). NORTH CAROLINA: Hayesville, VI–24–54 (BH–1).

Chrysops pikei Whit. TENNESSEE: Camden, VII–2–54 (C–1), VII–7–54 (C–1). ALABAMA: Mooresville, V–9–41 (1); Decatur, VI–11–41 (1); Greenbrier, VI–18–41 (1); Wilson Dam, VI–15–42 (1); Florence, V–28–53 (C–1).

Chrysops pudica O. S. TENNESSEE: Camden, VI–18–54 (C–1), VII–7–54 (C–1). ALABAMA: Somerville, VI–10–42 (1); Wilson Dam, VI–15–42 (1).

Chrysops striata O. S. TENNESSEE: Big Sandy, VII–28–54 (C–1); Paris, VIII–26–54 (S–1).

Chrysops univittata Macq. TENNESSEE: Paris, VI–12–51 (C–2), VI–17–54 (BU–1), VI–24–54 (C–1); Carderview, VI–16–53 (BM–1); Pickwick Dam Village, VI–23–53 (BM–1); Hustburg, VI–23–54 (C–1); Faxon, VI–24–54 (C–1); McKinnon, VI–24–54 (C–1); Camden, VII–2–54 (C–1). ALABAMA: Somerville, VI–10–42 (1); Rogersville, VI–4–54 (BM–1). NORTH CAROLINA: Hayesville, VI–24–54 (BH–2); Andrews, VII–7–54 (S–2). Of all deer flies considered in this report, *C. univittata* showed the widest range of habitat conditions. In the tributaries of the storage reservoirs up to 2,000′ in the Smoky Mountains, females were taken along mountain streams where mountain laurel and rhododendron were common plant species. In west Tennessee characteristic habitats appeared to be along gravel bottom streams in low hill country.

Chrysops upsilon Philip. ALABAMA: Decatur, VI–9–41 (12), VI–11–41 (15), VI–21–41 (9), VI–26–41 (1), VII–9–41 (3), VII–13–41 (4); Florence, VII–1–41 (1); Greenbrier, VII–26–55 (C–1), VIII–5–55 (C–1), VIII–19–55 (C–2).

Chrysops vittata Wied. TENNESSEE: Englewood, VII–9–52 (BM–3); Chattanooga, VI–2–53 (BM–1), VI–7–54 (BM–1); Paris, VI–23–54 (S–2), VII–8–54 (S–14), VIII–26–54 (S–27); Big Sandy, VIII–5–54 (C–1). ALABAMA: Decatur, VI–9–41 (3), VI–11–41 (6), VI–21–41 (3), VI–26–41 (1). NORTH CAROLINA: Hayesville, VI–24–54 (BH–5). In the mountainous area around Hayesville, N. C., deer flies have been considered a major pest to man and livestock in June. Adults of *C. vittata* were especially active along the wooded hill tops rather than in

pastured valleys. Near Paris, Tenn., this species was very abundant in a sandy creek bottom which had become swampy and colonized by alder (*Alnus rugosa*) due to interruption of drainage by highway construction.

Chrysops wiedemanni Kr. Tennessee: Bristol, VIII–9–51 (C–1); Camden, VI–23–53 (C–3), VIII–3–53 (BM–3), VIII–14–53 (C–1), VIII–15–53 (C–1), VII–1–54 (C–1), VII–2–54 (C–1), VII–7–54 (C–4), VII–27–54 (C–1), VIII–25–54 (C–1), VIII–26–54 (S–2) (C–1); Eva, VII–30–53 (BM–2); Hollow Rock, VIII–21–53 (C–2); Big Sandy, VII–28–54 (C–1); Paris, VIII–26–54 (S–1), IX–9–54 (S–1). Alabama: Decatur, VI–9–41 (4), VI–11–41 (3), VI–26–41 (1); Florence, VII–1–41 (2); Greenbrier, VI–20–55 (C–1), VII–14–55 (RT–1), VII–26–55 (C–1), VII–27–55 (C–1). Mississippi: Iuka, IX–22–52 (BM–1). This species was commonly found biting man in the major reservoir bottoms in north Alabama and West Tennessee with *C. flavida*.

Hamatabanus carolinensis (Macq.). Tennessee: Camden, V–10–54 (C–1). Alabama: Wilson Dam, VI–6–41 (1).

Leucotabanus annulatus (Say). Tennessee: Hustburg, VII–2–53 (LT–1); Parksville, VIII–27–53 (C–1); Tallassee, VII–6–54 (C–1); Tazewell, VII–6–54 (C–1); Camden, VII–7–54 (C–2); Knoxville, VII–15–55 (C–1). Alabama: Riverton, XII–1–54 (Larva-tree cavity–1), V–24–55 (Larvae-tree cavity–1); Waterloo, IV–7–55 (Larva-tree cavity–1), IX–5–55 (Larva–stump–1); Town Creek, VI–1–55 (Larvae–stump–3), VII–8–55 (pupa–stump–1). Adults of this species are active during the day and at night. Larvae and pupae were collected in the field from tree cavities and stump holes. Only one or two individuals were found in the cavity at one time. The larvae appeared more frequently in the moist wall of the cavity than in open water.

Tabanus americanus Forst. Tennessee: Camden, VII–29–54 (C–1). Alabama: Waterloo, VII–19–54 (C–1).

Tabanus atratus Fab. Tennessee: Dentville, VII–7–52 (BC–1); Camden, VII–7–53 (BM–1); Newport, IX–2–54 (BC–1); Lake City, VIII–8–55 (Barn–1); Hustburg, IX–15–55 (C–1). Alabama: Athens, VII–6–41 (1), VII–9–41 (2); Wilson Dam, VIII–2–41 (1); Sheffield, VII–11–53 (window–1); Rogersville, VIII–10–55 (C–1).

Tabanus calens L. Tennessee: Camden, VIII–14–53 (C–2), VIII–15–53 (C–2); Hollow Rock, VIII–21–53 (C–3); Paris, VIII–21–53 (BC–2); Perryville, VIII–19–55 (C–1). Alabama: Florence, VIII–27–41 (1); Greenbrier, VIII–29–55 (C–1) (BU–1).

Tabanus cinctus Fab. Tennessee: Tellico Plains, VI–25–52 (BC–1); Parksville, VI–24–54 (BU–3).

Tabanus cymatophorus O. S. Tennessee: Sugar Tree, VII–30–53 (C–1).

Tabanus difficilis Wied. Tennessee: Oak Ridge, VII–23–49 (S–1); Carderview, VI–9–54 (BH–3); Tazewell, V–31–55 (C–2).

Tabanus equalis Hine. Tennessee: Samburg, VI–18–52 (C–1); Hustburg, VIII–2–53 (LT–1); Camden, VII–7–54 (C–1).

Tabanus fulvulus Wied. Tennessee: Camden, VI–10–51 (C–2), VI–11–51 (C–1), V–10–54 (C–1); Samburg, VI–18–52 (C–1); Athens, VI–24–52 (C–1); McKinnon, VI–24–54 (C–1); Parksville, VI–24–54 (BU–1). Alabama: Wilson Dam, V–28–41 (2); Somerville, VI–10–41 (1). North Carolina: Hayesville, VI–24–54 (BH–1). Not observed feeding on man in the original studies, but one of the authors later (June 12, 1956) captured a specimen at Florence, Alabama, while it was attempting to feed on his arm.

Tabanus fulvulus pallidescens Philip. Tennessee: Camden, VI–15–51 (C–1), VII–6–53 (C–2); Parksville, VI–24–54 (BU–2). Alabama: Wilson Dam, V–22–41 (4), VI–6–41 (3), VI–15–41 (2); Somerville, VI–10–41 (1); Greenbrier, VI–26–41 (2).

Tabanus lasiophthalmus Macq. Tennessee: Samburg, VI–18–52 (C–1); Bristol, VI–10–54 (C–1). Alabama: Wilson Dam, V–22–41 (2), VI–11–41 (1); Decatur, V–27–41 (1).

Tabanus lineola Fab. TENNESSEE: Oak Ridge, VII–29–49 (S–1); Camden, VI–10–51 (C–1), VI–14–51 (C–11), VI–15–51 (C–2), VI–16–51 (Tent–10), V–21–53 (C–1), VI–9–53 (C–2), VII–6–53 (C–6), VIII–3–53 (C–1), VIII–14–53 (C–2), VIII–22–53 (C–1), IX–3–53 (C–1), VI–22–54 (C–1), VII–7–54 (C–1), VII–29–54 (C–1), VIII–26–54 (C–3), IX–10–54 (C–1); Sugar Tree, V–29–53 (C–1), VI–17–54 (C–1), VII–8–54 (C–1), VI–24–55 (C–1); Hustburg, VII–7–53 (LT–2), VII–14–53 (LT–1), VII–16–53 (LT–2), VII–30–53 (LT–1), VIII–1–53 (LT–1), VIII–6–53 (LT–5), VIII–11–53 (LT–1), VIII–19–54 (C–1), VIII–27–54 (C–1); Newport, VIII–14–53 (LT–1); Linden, V–20–54 (BU–1); McKinnon, VI–24–54 (C–2); Paris, VI–24–54 (C–1), VII–20–54 (C–1); Samburg, VII–22–54 (C–2), VII–23–54 (LT–1). ALABAMA: Decatur, V–27–41 (1); Wilson Dam, VI–9–41 (2); Somerville, VI–10–41 (1); Greenbrier, VI–18–41 (1); Florence, VII–15–53 (C–5); Rogersville, VII–2–54 (C–1); Madison, VIII–25–54 (C–1). This was the most common horse fly attacking livestock in the main reservoir bottoms of the Tennessee Valley.

Tabanus lineola scutellaris Walk. TENNESSEE: Oak Ridge, VII–23–49 (S–1).

Tabanus longiusculus Hine. TENNESSEE: Hollow Rock, VIII–21–53 (C–1); Camden, VIII–23–53 (C–1).

Tabanus melanocerus Wied. TENNESSEE: Camden, VII– –50 (C–1), VII–6–53 (C–3), VI–14–54 (C–1), VII–7–54 (C–1); Parksville, VI–24–54 (BU–1); Paris, VII–20–54 (C–1); Sugar Tree, VI–24–55 (C–1). ALABAMA: Waterloo, VII–19–54 (C–1). GEORGIA: Blairsville, VI–17–55 (C–1).

Tabanus molestus Say. TENNESSEE: Samburg, VI–15–51 (C–1), VI–18–52 (C–1); Tellico Plains, VI–25–52 (BC–1); Paris, VI–24–54 (C–1). ALABAMA: Wilson Dam, VI–16–41 (1), VII–12–42 (1).

Tabanus molestus mixis Philip. TENNESSEE: Camden, VI–15–51 (C–1), VI–19–51 (C–2); Paris, VI–3–54 (C–1), VI–24–54 (C–1); Parksville, VI–24–54 (BU–1). ALABAMA: Wilson Dam, V–24–53 (window–1). NORTH CAROLINA: Cherokee, V–25–54 (BU–1).

Tabanus mularis Stone. TENNESSEE: Paris, VI–12–51 (C–1); Chattanooga, VI–2–53 (C–1); Camden, VI–9–53 (C–1), VIII–14–53 (C–4), VIII–15–53 (C–1), VIII–22–53 (C–1); Sugar Tree, VII–3–53 (C–1); Big Sandy, VI–8–54 (window–1). ALABAMA: Decatur, VII–30–41 (1); Florence, VI–12–53 (C–4), VI–29–54 (C–1); Rogersville, VI–18–54 (C–1); Town Creek, VII–9–55 (C–1).

Tabanus nigrescens P. de B. ALABAMA: Scottsboro, VII–20–54 (C–1).

Tabanus nigripes Wied. TENNESSEE: Erwin, VII–7–54 (BH–1).

Tabanus petiolatus Hine. TENNESSEE: Camden, VI–22–54 (C–1), VIII–26–54 (C–1). ALABAMA: Decatur, VI–11–41 (1).

Tabanus proximus Walk. TENNESSEE: Sugar Tree, VIII–15–53 (C–1). ALABAMA: Greenbrier, VIII–29–55 (C–1).

Tabanus pumilus Macq. TENNESSEE: Oak Ridge, VII–23–49 (S–1); Sugar Tree, V–29–53 (C–1); Springville, V–25–55 (Helicopter–1).

Tabanus quinquevittatus Wied. TENNESSEE: Camden, VI–14–51 (C–1), VIII–3–53 (BM–1), VIII–6–53 (C–3), VIII–14–53 (C–11), VIII–15–53 (C–1), VIII–22–53 (C–3), VII–29–54 (C–1), VIII–4–54 (C–1); Sugar Tree, VI–14–53 (C–1), VI–24–53 (C–1); McKinnon, VII–30–53 (C–1), VIII–4–53 (BM–1); Big Sandy, VIII–5–54 (C–1); Hustburg, VIII–19–54 (C–1). Of the 36 species of horse flies taken in the field, only *T. quinquevittatus* was found actually feeding on man (but see note on *T. fulvulus*). They appeared in west Tennessee along flat reservoir margins where buttonball was the dominant plant species.

Tabanus reinwardtii Wied. TENNESSEE: Carderview, VII–16–52 (C–1).

Tabanus sackeni Fair. TENNESSEE: Carderview, VII–16–52 (Under Bridge–1); Camden, VIII–15–53 (C–1).

Tabanus sparus Whit. ALABAMA: Wilson Dam, V–27–42 (1); Triana, V–28–42 (1).

Tabanus sparus milleri Whit. TEN-

nessee: Oak Ridge, VII–23–49 (S–1); Camden, VI–8–53 (C–1), VII–6–53 (C–1), VIII–3–53 (C–1), VI–22–54 (C–1), VII–7–54 (C–1), V–25–55 (C–1); Norris, VI–19–53 (C–1); Parksville, VI–19–53 (C–1); Eva, VII–30–53 (C–1); McKinnon, VI–24–54 (C–1), VI–28–54 (C–1); Sugar Tree, VII–8–54 (C–1); Springville, V–25–55 (Helicopter–3); Jasper, VI–17–55 (C–1). Alabama: Decatur, VI–11–41 (2), VI–26–41 (1), VII–20–54 (Helicopter–1); Athens, VII–6–41 (1). North Carolina: Hayesville, VI–24–54 (BH–1); Andrews, VII–7–54 (C–2).

Tabanus stygius Say. Alabama: Wilson Dam, VII–1–41 (1), V–26–42 (1).

Tabanus sulcifrons Macq. Tennessee: Oak Ridge, VII–15–49 (S–1); Camden, VI–14–51 (C–1), VII–7–53 (BM–1), VIII–14–53 (C–1), VIII–15–53 (C–1), VIII–22–53 (C–6) (Log–1), VII–29–54 (C–2), VIII–19–54 (C–1); Bristol, VIII–9–51 (C–1); Tellico Plains, VI–25–52 (BC–1); Newport, VII–1–53 (Culvert–2), VII–14–53 (Slough Bank–1), VII–9–54 (C–3), IX–2–54 (BC–1); Sugar Tree, VII–3–53 (C–1); Paris, VIII–22–53 (C–1), IX–3–53 (BC–1), VIII–19–55 (C–1); Parksville, VIII–27–53 (C–1), VI–24–54 (BU–10); Lobelville, VI–24–54 (BU–1); Erwin, VII–7–54 (BH–1); McKinnon, VII–14–54 (C–1); Big Sandy, VII–28–54 (C–1); Pickwick Dam Village, VIII–4–54 (C–1); Rogersville, IX–24–54 (BH–1); Lake City, VII–14–55 (C–1), VII–29–55 (C–1); New Tazewell, VII–25–55 (Barn–1). Alabama: Triana, VIII–29–41 (2); Wilson Dam, VI–26–42 (1), VI–30–53 (Window–2), IX–10–53 (C–1), VI–25–54 (BM–1); Florence, VIII–20–53 (C–1), VIII–12–54 (C–1); Waterloo, IX–18–53 (BU–1). North Carolina: Bryson City, VII–1–53 (C–1). This species is widespread in the Tennessee Valley and commonly encountered in the river bottoms.

Tabanus trimaculatus P. de B. Tennessee: Oak Ridge, VII–23–49 (S–1); Camden, VI–14–51 (C–1), VI–22–54 (C–1), VII–7–54 (C–1); Sugar Tree, V–29–53 (C–1); Paris, V–28–54 (C–1), V–26–55 (C–1); Athens, VI–16–55 (C–1). Alabama: Wilson Dam, VI–15–42 (1); Rogersville, VI–18–54 (C–1).

Tabanus trispilus Wied. Tennessee: Tellico Plains, VI–25–52 (BC–3).

Tabanus turbidus Wied. Tennessee: Perryville, V–25–55 (C–1); Hustburg, VI–22–55 (C–6) (BP–4); Springville, VI–22–55 (C–2). Alabama: Wilson Dam, V–26–41 (1); Sheffield, VII–13–54 (C–1).

Tabanus venustus O. S. Tennessee: Perryville, VII–12–55 (C–1).

Tabanus vittiger schwardti Philip. Tennessee: Samburg, VIII–23–41 (2); Perryville, VII–12–55 (C–1). Alabama: Decatur, VIII–13–41 (1); Leighton, VIII–18–41 (1).

Reference

Pechuman, L. L. 1954. Seasonal distribution of some Tennessee Tabaninae (Diptera, Tabanidae). Bull. Brook. Ent. Soc. 49(5):128–130.

PLEASE CORRECT

On the cover of Vol. 16, No. 4 (December 1956), of *Mosquito News* in the Table of Contents, the senior authorship of the paper, "A Population Study of the *Culicoides* Midges of the Edwards Plateau Region of Texas," was wrongly ascribed to W. W. Smith. We regret this error and take this opportunity to call attention to the fact that the names of the authors should read "W. W. Wirth and L. J. Bottimer." The authorship is correctly stated where the paper appears in the text.

MIAMI

AMERICAN MOSQUITO CONTROL ASSOCIATION

April 28–May 2, 1957

THIRTEENTH ANNUAL MEETING

Convening Jointly with

FLORIDA ANTI-MOSQUITO ASSOCIATION

Rates

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	$8.00	$12.00 Side Rooms
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Before April 1, 1957

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Includes: Night's lodging; all fishing tackle and bait; preparation of fish to take home; visit to Old Fort and Prison where Dr. Mudd, famous doctor who treated Lincoln's assassin, was imprisoned.

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Leave Miami Friday Morning, May 3

EDITORIAL

LEST WE FORGET!

We are duly, and we believe justly, proud of the American Mosquito Control Association, the substantial accomplishments it has achieved, and the solidarity among mosquito workers which it has engendered.

But long before our time, on January 28, 1904, to be exact, there was formed the "National Mosquito Extermination Society," with officers as follows:

Chairman—William J. Matheson, N. Y. & Long Island (Pres., W. J. Matheson Co.)

Vice Chairman—Walter C. Kerr, N. Y. & Staten Island (Pres., Westinghouse, Church, Kerr & Co.)

Secretary—Henry Clay Weeks, Bayside, L. I. (Engineer in Economics)

Treasurer—Frederick C. Beach, N. Y. (Editor, *Scientific American*).

An Executive Committee of five persons was named, there was created an Advisory Board of 27 men and one woman (mostly selected from the list of those who had been named as vice-presidents at a convention which had been held in New York on December 16, 1903 (See printed report "Proceedings of The First General Convention to Consider the Questions Involved in Mosquito Extermination," published at Brooklyn, New York, 1904). There was also formed an Advisory Board of Entomologists, consisting of 25 persons, some of whom were state entomologists of the states particularly concerned, the others being men who had made mosquitoes a special study. The list included such famaliar names as Dr. L. O. Howard, Washington; Dr. E. Porter Felt, Albany, New York; Dr. John B. Smith, New Brunswick, New Jersey; Prof. W. E. Britton, New Haven, Connecticut; Prof. V. L. Kellogg, Stanford University, California; Prof. Herbert Osborn, Columbus, Ohio; Prof. E. D. Sanderson, College Station, Texas; Dr. D. W. Coquillett, Washington, D. C.; Dr. H. C. Dyar, Washington, D. C.; Prof. Glenn W. Herrick, Mississippi; and others.

Memberships were provided for, with annual dues, as follows: Active—$2.00, Associate—$5.00, Benefactor—$25.00, Life Member—$100.00, Patron—$500.00, Founder—$1000.00. It is not recorded that there ever were any Patrons, or Founders! But in the Proceedings of the Second Anti-Mosquito Convention, December 15 and 16, 1904, there is record of 4 Benefactors and 1 Life Member!

During the second convention, at which some very good papers were presented, a dinner was given to out-of-town participants at the New York Yacht Club by one Mr. George C. Rand. The guests, in appreciation, inscribed "A Note of Prophecy" to their host. Among the many signers was one William Lyman Underwood of Boston, who wrote this verse, appropriate to all of us, even today:

"How doth the pesky 'skeeter'
  Employ each stealthy minute?
She gathers blood from man to man,
  In fact, she revels in it.
For this reason, let us not forget, we have
  Combined to do them up."

This is on one of the final pages of the report of the second convention—and this reporter has no record of any further doings of this group! [Do any of our readers have any information on the subsequent history of this group as an organization?]

—THOMAS D. MULHERN.

NEWS AND NOTES

The Twenty-Fifth Annual Conference of the California Mosquito Control Association drew so many folks from all over that it seemed almost like a curtain raiser for the AMCA meetings to come next month. Uncle Harry Stage had probably come about as far as anyone, although the last leg of his trip was just down from his new home in Oregon, but most of us hadn't seen him since way back yonder whilst he has been gallivanting around the world. He hasn't changed a bit, we need hardly add, since he hasn't changed a bit in the more than 20 years *we've* known him. Also from afar, were Bob Vannote, who brought the Word from New Jersey, and Otto McFeely, AMCA's well-known member from Illinois. Oregon, in addition to sending Harry, sent Milt Buehler, C. M. Gjullin, Gaines Eddy, Jack Huber, Vern Miller, Gene Fields, all AMCA members, and Fred Lewis and Gene Albert, who we hope will be. Utah was represented by Don Rees, Glen Collett, Russ Anderson, Sam Harman, Jay Graham, this year's President of the Utah Mosquito Control Association, and Charles Geurts. Roy Fritz came up from Mexico City and Archie Hess represented CDC, bringing Dr. S. W. Simmons, who was one of the feature speakers, as was Dr. Art Lindquist, from Washington, D. C. Helen Louise Durkee came down, too, from that happy land of her own she's been sequestering herself in, and stayed far too short a time for all her friends' pleasure. Tommy Mulhern took pictures (and gave some interesting talks on equipment and stuff) and Ted Raley begged everyone to remember the AMCA meetings in Miami. Harold Gray came looking mighty fit, as everyone was glad to see after the bouts he's had with ill health, and proved his enduring vigor by delivering a mighty fine address at one meeting and moderating another. And Much More made up two and a half jam-packed days, which we can't even begin to cover.

The 1957 Officers of the C.M.C.A., by the way, are Howard Greenfield, President, Bob Portman, Vice-President, and Ed Washburn, Secretary-Treasurer, while the regional representatives are Jack Kimball, Southern California, Ed Davis, San Joaquin Valley, Paul Jones, Bay Area, and Joe Willis, Sacramento Valley.

In Connection with All of which We Can Now Announce that Harry Stage's new address is 2235 N.E. 121st Avenue, Portland 20, Oregon, and he hopes that all AMCA members in the vicinity will drop in and all those not in the vicinity will write to him. Though with all the Kodachrome slides he has of all those exotic places he's been in lately, he may be off any moment on a lecture tour.

And May We Remind You that the Utah Mosquito Control Association is having its meeting at Salt Lake City on the 8th and 9th of March, at the University campus. Bob Vannote announced that the New Jersey Mosquito Control Association meetings will be held in March, at the Chalfonte-Haddon Hall in Atlantic City, and the Virginia Association meetings were held in Virginia Beach in late February.

Which May be a Good Place to Insert a Little Plea for News and Notes, which we will lead into with a plaintive query, "Don't you guys love us any more?" We used to get notices of lots of meetings and things and someone would send us a quick pencil note about what went on at them, even sometimes we got a regular typed-up report! How about it, fellers? Let us in on your doings, huh? Lot's of the folks would like to know what you're doing!!

Rollie Dorer's Virginia Bureau of Insect and Rodent Control announces that it has gotten itself all decked out in a new building at 5709 Sellger Drive, Norfolk 2, Virginia. The building is close to Route 13 and just one mile south of the Virginia Beach Blvd (Route 58), and all friends "are invited to stop in and inspect these modern and convenient quarters." A tip to those who may be driving South to the meetings in Miami in April!

Virginia's Mosquito Control Districts now number fifteen, having recently welcomed into their group Washington District, in Norfolk County. The new district not only voted itself into existence but also voted 10¢ per hundred dollars of its tax valuation as a special mosquito control tax.

Don Pletsch Apparently Just Uses His Official Addresses as Spring Boards, for while he was "in" Taiwan we kept hearing of him in a dozen other fascinating Oriental places and now that he's "in" Mexico City, we got word at Christmas that he was on a special project in Jamaica. We hope for further word as to whether that famous rum and ginger makes the mosquitoes stouter or if it fills them with a never-mind feeling . . . or perhaps it adds to their insecticide resistance? Don also wrote that the new total coverage program in Mexico was due to start 2 January, and Roy Fritz added at the California meetings that they were having very encouraging results down South of the Border.

Wilbur Mitchell, Formerly with the Freeport (Illinois) Mosquito Abatement District and Now Technical Director of the Northwest Mosquito Abatement District of Cook County, Illinois, sends us the First Annual Report of that new member of our family of mosquito abatement districts. This District, which was overwhelmingly voted into existence on 10 April, 1956, includes some 235 square

miles in the townships of Wheeling, Palatine, Barrington, Hanover, Schaumburg, Elk Grove, Maine and a small part of Northfield. A large number of civic minded individuals worked without compensation to complete the requirements in forming the District and so much interest was aroused that nearby areas are expected to annex themselves to the District in the near future. The first season's operations were largely concerned with aerial and ground survey and mapping, with assembling the required equipment and investigating the feasibility of pre-hatch treatments and similar programming activities. In their first report, the District also acknowledged their gratitude to AMCA members FRANKLIN C. WRAY, J. D. WILLIAMS and W. J. BUCHANAN, for their advice and guidance.

MILT BUEHLER HAS FOR THE FOURTH TIME ISSUED HIS USUAL INFORMATIVE AND COMPREHENSIVE ANNUAL REPORT FOR LANE COUNTY, OREGON, in which he gives a thorough discussion of the bionomics and ecological factors involved in mosquito control in his area. Of interest to other mosquito control workers, are the several tables giving complete breakdowns on operation costs, materials used, etc. Milt ran head-on into resistance to DDT in one of his areas, this year, and has intensified the source-reduction aspects of his program, to which, in common with everyone else, he has been giving more and more attention anyway.

LES BRUMBAUGH AND CHET ROBINSON JOINED IN REPORTING (WITH A MIXTURE OF SADNESS AND GLEE) THAT SAN JOAQUIN AND ALAMEDA, CALIFORNIA, MOSQUITO ABATEMENT DISTRICTS had had the least precipitation in years. While this was good news for mosquito abatement it was a little rough on the farmers, some of whom had to turn to extensive and unseasonable irrigation. Temperatures at night were quite low, however, and moderately so during the day, and mosquito production was not measurably increased by the artificial watering. Both Districts emphasized source reduction and Chet noted a considerable reduction in the number of service requests, except in December when he had a total of two. This was unfortunately a one hundred percent increase over last year. Very discouraging.

TED RALEY'S MEMORANDUM, which was received too late to note in December, contained ten points so important to the membership that we are going to take the liberty of reprinting it here. Noting the good health of our Association and the fact that *Mosquito News* was sent to 1281 accounts in September as compared with only 1136 last year, Ted reiterated the value of Good Neighbor accounts and urged all members to exert their influence, not only to enlarge the Good Neighbor membership but also to reduce further our already small number of cancellations and delinquencies. Ted's Ten Points were as follows:

1. New members and *Mosquito News* subscriptions
2. Adoption of a Good Neighbor
3. Securing of an advertisement for *Mosquito News*
4. Promotion of sale of Bulletins
5. Locating and sending to Walt Henderson, back issues of *Mosquito News*
6. Making suggestions for Bylaws revision to Bailey Pepper
7. Paying 1957 dues NOW
8. Making a contribution to the "Operational and Scientific Notes" section of *Mosquito News*
9. Getting approval for travel to Miami Beach, April 28–May 2
10. Urging everyone else to attend the Florida meeting, too.

To which we would like to add one more Thing To Do Now: Send in News and Notes on your District's activities, on yourself and on those you know in AMCA, to NEWS AND NOTES, c/o *DD 111*, District Public Works Office, San Bruno, California.

A VALUABLE REFERENCE BOOK ON EXCAVATING AND FILLING AND THE EQUIPMENT TO DO IT WITH is reviewed in "California Vector Views" by Ralph J. Black. The book, entitled "Moving the Earth," is by Herbert L. Nichols, Jr., and is published by North Castle Books, Greenwich, Connecticut. It costs $15.00 but is well worth it in the opinion of the reviewer, who says, "Mosquito abatement workers will find the answers to many common problems in the discussions of agricultural grading, ponds and dewatering. All the important types of excavating, hauling, and grading equipment are described in detail in Part Two. The characteristics and peculiarities of each type of equipment are evaluated for almost every conceivable job. Constant attention also is given to problems of maintenance and repair. . . ."

WHO'S WHO IN THE AMERICAN MOSQUITO CONTROL ASSOCIATION COVERS A WIDE RANGE OF ACTIVITIES AND AS A SAMPLE WE BRING YOU THE FOLLOWING:

DR. LAFE R. EDMUNDS, for instance, who has recently joined the U. S. Army Corps of Engineers Research and Development Laboratories, at Ft. Belvoir, Va., has been a professor of entomology at Mississippi State College. He was born in Salt Lake City, Utah, (the "R" is for Rees, by the way) in 1924, receiving his B.S. there and his Ph.D. from Ohio State. Just before those events he had been in Uncle Sam's Infantry, which may have influenced his future interests a little. He also served a hitch with the U. S. Public Health Service before going to Mississippi and says his hobbies are insect biology, conservation and hunting.

DR. TERUHIKO HOSOI, of the Tokyo Institute of Technology, in Tokyo, whose comments on the

progress of mosquito work in Japan appeared in N and N last September, became well known as a friend and fellow-worker to many Americans concerned with mosquito control in Japan immediately after the war, when he was with the Metropolitan Department of Health. Dr. Hosoi, who was born in 1910, graduated in 1933 from the Imperial University in Tokyo, receiving his degree in zoology, and three years later completed his graduate studies at the same University. He was with the Institute for Infectious Diseases of the University for three years, working under Drs. Yamada and Mitamura on the study of Japanese B encephalitis and subsequently made a malaria survey on the middle Yangtse River. In 1950 he joined the U. S. Army 406th Medical General Laboratory in Tokyo, as Research Associate, and in 1954 went to his present position as Professor at the Tokyo Institute of Technology.

Dr. Nanzaburo Omori, another of our foreign members, who is Professor and Director of the Department of Medical Zoology at Nagasaki University, is a graduate of the Department of Entomology of the Kyoto Imperial University, and spent fifteen years on Formosa, where he was engaged in ecological studies on the Formosan anophelines at the Institute for Tropical Medicine of the University of Taiwan. Since 1951, he has been engaged in studying the *pipiens* complex, the role of mosquitoes in the transmission of filariasis, and related subjects, and states that his hobby is "Go," a highly complicated Japanese form of checkers mixed with an amplified version of tic-tac-toe and played with what seems to the uninitiated like hundreds of counters on a board crossed by nineteen lines each way to form a total of *361* intersections. The resulting traffic pattern is unimaginably intricate and we can only bow our heads in reverence before anyone who can even do it, let alone enjoy it.

Dr. Alan Stone keeps track of equally intricate matters in a mind which encompasses the characters of virtually every mosquito ever captured and recorded. Born in Brooklyn in 1904, Dr. Stone received his B.Sc. from Cornell in 1926 and his doctorate three years later. He taught for two years at Dartmouth College and joined the Division of Insect Detection and Identification in 1931 in its former incarnation as part of the U. S. National Museum, where it still is. Alan was too modest to put much info on his form but by a little peeking into American Men of Science, delving of memory, etc., we can add that he is a member of the Entomological Society of America, of which he was a Fellow prior to the amalgamation of the two entomological societies, that he is also a member of the Washington Entomological Society, of which he was President in 1951, and of the Washington Academy of Science. His hobby is said to be the identification of Diptera, and as we mentioned some time back, he can have little time for anything else, since he and Ken Knight are spending nearly every non-daylight hour on the side task of compiling a catalogue of the mosquitoes of the world, something which has not been done since 1931 and, as we all are aware, is badly needed.

Dr. Bertram I. Gerry, of the Massachusetts State Reclamation Board, and long-time foe of mosquitoes in the Bay State, was born (it says here in American Men of Science) in Salem in 1896, and received his doctor's degree from Harvard in 1931. After instructing at Tufts College for some six years, he joined the Massachusetts Department of Agriculture and began putting their feet on the right path in insect control. He has been active in the establishment and continuation of mosquito control in the New England States and has often contributed welcome bits of information to keep the rest of us posted on what goes on up there. His hobby is Horticulture and his entomological interests, besides mosquitoes, embrace also the related problems of the Tabanidae and the Heleidae.

Ken Quarterman, presently Chief of the Technical Development Laboratories of the CDC, U. S. Public Health Service, at Savannah, Georgia, was born in that city in 1912 and received his degree in zoology and entomology at Mississippi State College and his Master of Public Health from Johns Hopkins. He worked with the U. S. Department of Agriculture's long-time Bureau of Entomology and Plant Quarantine and transferred to Public Health Service in 1947. Ken lists his hobbies as hunting, fishing and photography, all of which can be readily understood by anyone who has visited Savannah, surrounded as it is by natural wildlife areas and filled as it is with azaleas, Southern charm and beautiful women. (Ken didn't say his hobby *wasn't* taking pictures of lovely ladies, and we bet he takes a little old practice shot now and then.)

Now That the American Mosquito Control Association Has Completed Fifteen Years of Vigorous Existence, how many of our readers can remember the names of our past presidents? Of course, maybe it isn't quite fifteen years, if you don't count the first three years as the Eastern Association of Mosquito Control Workers, but we think that formative period was an integral part of our birth and growth. Well, fellow members, here's the roster, and a grand one it is. Some of the men have passed on, most of them are still in there giving our Association strength. All of them have our warmest admiration and deepest gratitude for a hard job, well done.

1941–43	L. A. Stearns
1943	R. C. Botsford
1944	R. L. Vannote
1945	Perry W. Ruth
1946	Harry H. Stage
1947	Harry H. Stage
1948	Duke Peters

1949	Harold Gray
1950	Lester Smith
1951	Don M. Rees
1952	C. R. Twinn
1953	F. C. Bishopp
1954	Rollie Dorer
1955	Dick Peters
1956	Fred Stutz
1957	Art Lindquist, coming up

Yes, admiration, and gratitude . . . and pride!

W. A. McDONALD, WHO IS A NEW A.M.C.A. MEMBER AND ASSISTED IN STUDIES AT U.C.L.A. on California *Anopheles, Uranotaenia* and *Aedes*, has recently reported in from Nigeria, where he is now assigned. He writes, "We find the climate here very similar to California's, at least in the dry season—hot, dry days and cool (often cold; last night was 52° F.) nights; the rains will start in April–May and last until September; this area gets about 50 inches on the average. Kaduna, being the capitol of the Northern Region, has practically all the facilities of any large American or English town and is expanding rapidly.

"Mosquito-wise, *Aedes aegypti* breeds locally and various *Anopheles* were common evening visitors during October–November, but have virtually disappeared with the advent of the cold weather. *Culex, Anopheles* and *Uranotaenia* are found in a nearby tributary of the Kaduna River; I am currently rearing a batch of *Culex (Lutzia) tigripes* by feeding them the larvae of other species.

"While in Lagos in October, I was shown through the Malaria Service laboratories at Yaba by Dr. L. Bruce-Chwatt and Roy Elliott, who sends regards to the many people he met last year on his WHO tour of the U. S. I was amazed to learn that the entire program of training, research and pilot control projects is handled by an annual budget equivalent to about 50,000 U. S. dollars." We hope that member McDonald has a most successful tour in Nigeria and sends us more detailed comments from time to time on the scope and progress of his and the Malaria Service programs.

ANYBODY HERE FROM NEBRASKA? An unidentified friend in the Nebraska State Department of Health sends us an information sheet which contains some data we think might be of interest to others of us. In the fall of 1952, 1955 and 1956, questionnaires were sent to all municipalities in Nebraska, asking whether or not they had a Fly and Mosquito Control Program and what insecticide was used, together with the cost. Ninety-five cities reported an average cost per person of twenty-seven cents; 387,624 persons were protected by the programs of these cities. The total amount of money spent for an average year was given as $104,658.48 and the three most popular insecticides were (1) DDT, (2) chlordane and (3) malathion, presumably in the order listed. Perhaps members DERKSEN, KEENER and RAPP can find out for us what proportion of these programs was mosquito control and how much was fly control. It is interesting, incidentally, that the two are reported together, for many mosquito abatement districts are finding that they are being inexorably edged along toward the more inclusive programs of housefly and biting fly control.

UNDER THE HEADING "BUZZ-BOMBED!", MARVIN C. KRAMER, Asst. Manager of the Alameda County M.A.D., has sent in the accompanying photograph and information.

"At the risk of being accused of a provincialism almost as great as that in Texas, we want to report the size of the mosquitoes we are raising in California. The bomber in question landed in the Alameda County Mosquito Abatement District recently. No virus or protozoan has yet been isolated from it, but it is doubtful whether such a pathogen is necessary; it will scare you to death. Dimensions are 4½ feet from the tip of its wood drill proboscis to the tip of the abdomen, and a wing spread of 4 feet 2 inches. Its heart is an electric motor that moves its wings up and down at a beat of about 16 per minute. A flasher button makes its red eyes flash to a brighter color at frequent intervals. The sound of the motor gives the mosquito an ominous hum. The cuticula is a heavy, neoprene-treated nylon, covered with the elastic mesh used for covering fowl when preparing them for the freezer. This covering surrounds a framework of metal rods welded together at strategic points.

The legs are of copper tubing bent to shape and painted the appropriate colors. The wings are made of metal rods covered with clear plastic, on which the veins are painted. The markings suggest *Culex tarsalis*. After capturing this prize, we exhibited it by fastening a light chain to the top of the thorax and suspending it from a metal pipe standard made of ½ inch galvanized pipe.

"Construction of the model mosquito was prompted by a 'County Government on Display' exhibit at the Alameda County Courthouse in October, 1956, at which we had a booth. Mr. Robinson and the mosquito were featured on a Richfield Oil Company *Success Story* TV program coving the County exhibits. We decided to make it durable enough so it could be used for similar booths or parades in the future. We have had some inquiries regarding it from various sections, so in 'striving for increased efficiency' or because we are fundamentally lazy, we felt the *News* could serve a fine purpose in spreading the word so we wouldn't have to."

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D. B. Smith & Co., Inc XVII
Spraying Systems, Inc XI
Todd Shipyards Corp X
Utah Mosquito Abatement Assn XVI
Velsicol Corporation V
Virginia Mosquito Control Assn XVI
Willys Motors I
Wyco, Inc. XV

REVIEWS AND ABSTRACTS

THE EFFECTS OF KNOWN REPEATED ORAL DOSES OF CHLOROPHENOTHANE (DDT) IN MAN. W. J. Hayes, W. F. Durham, and C. Cueto, Jr. J. Amer. Med. Assoc. Vol. 162. Oct. 27, 1956. This report emanating from the Communicable Disease Center of the U. S. Public Health Service in Savannah, Georgia, concerns an experimental study of the storage and excretion of DDT in man, and the possible clinical effects of DDT given in many small daily doses. Based on the premise that DDT occurs regularly in prepared meals, and that it is stored in the fat of most persons in the general population, this research becomes of special interest to all mosquito control workers. After all, DDT continues as the predominant residual insecticide for public health use around the world. Publication of the report has been authorized by the Council on Pharmacy and Chemistry of the American Medical Association. It has therefore been reviewed critically by competent scientists and may be considered as having unusual merit.

Briefly, fifty-one volunteers were given daily oral doses of DDT at varying intervals, one-third receiving as much as 35 mg. DDT per man, per day, which, incidentally, is considered about 200 times the daily amount which an average man receives from his diet. During the study, no volunteer complained of any symptom or showed, by the tests used, any sign of illness that did not have an easily recognized cause clearly unrelated to exposure to DDT. A method for determining the concentration of DDA (bis (p-chlorophenyl) acetic acid) was also perfected. In general, the research indicates that a large safety factor is associated with DDT, as it now occurs in the general diet. Undoubtedly many readers of MOSQUITO NEWS will wish to study the data in greater detail. The reference can be obtained at almost any library, or, perhaps, a reprint can be had by writing the authors.—H. H. Stage.

AN ANATOMICAL AND HISTOLOGICAL STUDY OF THE FEMALE REPRODUCTIVE SYSTEM AND FOLLICULAR DEVELOPMENT IN *Aedes aegypti* (L.). James J. Parks, M.Sc. Thesis, Dept. of Entomol. and Econ. Zool., Univ. of Minnesota, 1955, pp. iii plus 72. This is a study of the female reproductive system and the development of the egg up to (but not including) fertilization. The results are based on dissections and sectional material. An introductory section reviews previous investigations relevant to the study and the section which follows explains in detail the methods used.

The anatomy and histology of the female reproductive system are discussed at length and illustrated by a photograph of a longitudinal section of the abdomen. The point is made that there is a flap blocking the passageway between the vagina and spermathecal duct which might interfere with artificial insemination of the female.

The histology of the ovary is described and illustrated from third and fourth instar larvae, pupae, and adults. In the adult the oocyte is within a follicle which develops for about 24 hours following emergence (27°C.) and then enters a "resting stage"; development will continue only after the mosquito has taken blood. Counts of oocyte nuclei of 6 ovaries from 3 newly emerged (teneral) females showed that there were 70 primary follicles and thus 70 ovarioles in each. This suggests a limit of 140 eggs for a single clutch of *aegypti* but as a rule less than a hundred eggs are laid.

After the mosquito has taken blood, about 60 hours are required for the development of the eggs till they are ready for laying. During this time the nucleus undergoes a fantastic development and as a result assumes the shape of a thin sheet which extends throughout the egg. The nucleus thus presents a maximum surface area to the yolk.

After the first clutch of eggs has been laid the ovary appears different which suggests that it might be possible to differentiate nulliparous and parous females by the appearance of the ovaries. —A Ralph Barr, Univ. of Kansas, Lawrence, Kansas.

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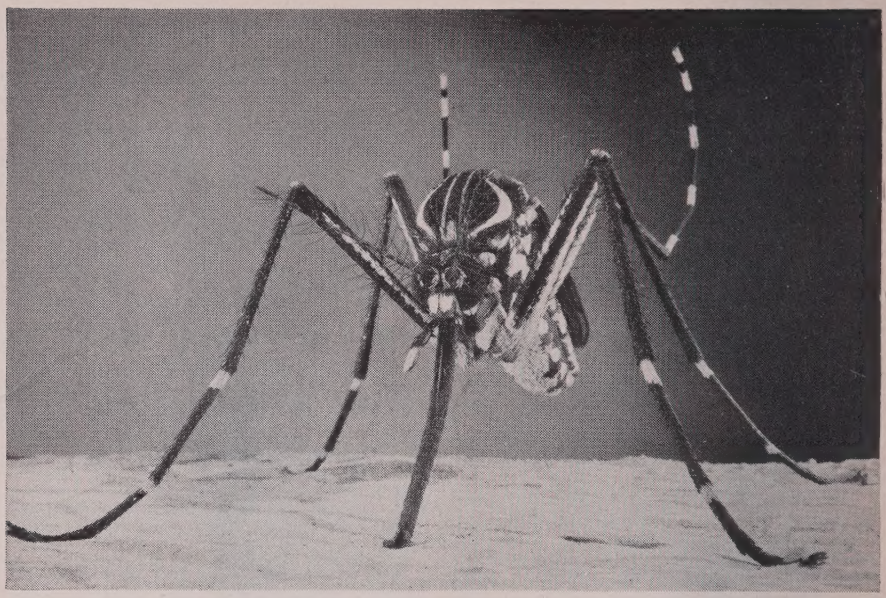

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